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Preface

The UJNR (The U.S.-Japan Cooperative Program in Natural Resources) Aquaculture Panel was established in 1968, and since 1971 the business meeting and symposium have been held every year. Through the long history of UJNR, Aquaculture Panel has contributed to development of aquaculture researches of both countries by means of various cooperative activities, i.e. the exchange of scientists, the exchange of literatures and promoting joint research projects. It is understood that the Aquaculture Panel is one of the most active UJNR panels by both countries.

In October 2010, the 39th Japan-U.S. Joint Meeting of the Aquaculture Panel was convened in Kagoshima and Miyazaki Prefectures. The present special issue of Bulletin of Fisheries Research Agency is the proceedings of the 39th UJNR Aquaculture Panel Symposium "The Present and Future of the Aquaculture Industry". This symposium organized by the Aquaculture Panel and Kagoshima University was held at Inamori Hall, Kagoshima University, under the 8th UJNR three-year plan.

The production of aquaculture in the world has increased steadily over the past two decades. Further development of aquaculture is highly desired as an important source of foods for human being all over the world. On this symposium, we focused on the problems faced by the aquaculture industries of the two nations and strategies and solutions to create competitive new and existing aquaculture industries. We also expected to highlight key bottlenecks that need to be addressed in future meeting. It is my great pleasure that the present UJNR proceedings containing high quality papers of the selected U.S. and Japanese aquaculture scientists will help understand the problems and future aspects of aquaculture industry.

Finally I would like to express my sincere gratitude to the colleagues involved in the UJNR Aquaculture Panel meeting for their efforts to prepare and organize the symposium. I also would like to deeply thank the editorial board members for publishing the proceedings.

Takaji Iida
Chair of UJNR Aquaculture Panel
Director General
National Research Institute of
Aquaculture
Fisheries Research Agency

CONTENTS

Preface

Contents

Program

Overview of Aquaculture Research Policy

1. Course of the Research for Sustainable Aquaculture in Japan
Fuminari Ito (National Research Institute of Aquaculture, Fisheries Research Agency) 1

Aquaculture Industry Overview and Research Planning

2. Aquaculture in the United States of America: Present Status and Future Opportunities
Paul G. Olin (UCSD, Scripps Institution of Oceanography) 7

Techniques of Aquaculture Production

3. Spawning and Larval Rearing of California Yellowtail (*Seriola lalandi*) in Southern California
Kevin Stuart (Hubbs-SeaWorld Research Institute) 15
4. Charting the Future of Aquaculture Feeds in the United States
Michael B. Rust (Resource Enhancement and Utilization Technologies Division, Northwest Fisheries Science Center, NOAA) 23
5. Progress of DNA Marker-Assisted Breeding in Mariculture Finfish
Akiyuki Ozaki (National Research Institute of Aquaculture, FRA) 31
6. Development and Evaluation of Real-Time Loop-Mediated Isothermal Amplification Methods for the Rapid Detection of Penaeid Viruses
Tohru Mekata (National Research Institute of Aquaculture, FRA) 39

Management, Social and Economic Issues of the Aquaculture Industry

7. The Political Economics of United States Marine Aquaculture
Gunnar Knapp (Institute of Social and Economic Research, University of Alaska Anchorage) 51

Supporting Techniques of Aquaculture

8. Inland Marine Fish Culture in Low-Salinity Recirculating Aquaculture Systems
Marty A. Riche (U.S. Dept. of Agriculture, Agricultural Research Service) 65
9. Land-Based Poly-Eco-Aquaculture of Abalone and Seaweed in a Small Scale Recirculating System Using a Recycled Freezer Container
Mohammad M. Rahman (Faculty of Fisheries, Kagoshima University) 77
10. Pond-to-Plate Analysis of the U.S. Farm-Raised Catfish Industry
Terrill R. Hanson (Department of Fisheries and Allied Aquacultures, Auburn University) 85

Stock Enhancement

11. Case Studies in Flatfish Stock Enhancement: A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage Conditioning for Japanese Flounder, *Paralichthys olivaceus*, in Wakasa Bay, Japan
Michelle L. Walsh (University of New Hampshire) 93
12. Acoustic Conditioning and Ranching of Black Sea Bass *Centropristis striata* in Massachusetts USA
Scott Lindell (Marine Resources Center, Marine Biological Laboratory) 103

Hatchery, the Present State and Issues

13. Artificial Completion of the Japanese Eel, *Anguilla japonica*, Life Cycle: Challenge to Mass Production
Yoshitsugu Masuda (Shibushi Laboratory, National Research Institute of Aquaculture, FRA) 111

CONTENTS

Preface
Contents
Abbreviations

Overview of Japanese Fisheries Policy

1. Current Status of Fisheries in Japan

1.1. Current Status of Fisheries in Japan

1.2. Current Status of Fisheries in Japan

1.3. Current Status of Fisheries in Japan

1.4. Current Status of Fisheries in Japan

1.5. Current Status of Fisheries in Japan

1.6. Current Status of Fisheries in Japan

1.7. Current Status of Fisheries in Japan

1.8. Current Status of Fisheries in Japan

1.9. Current Status of Fisheries in Japan

1.10. Current Status of Fisheries in Japan

1.11. Current Status of Fisheries in Japan

1.12. Current Status of Fisheries in Japan

1.13. Current Status of Fisheries in Japan

1.14. Current Status of Fisheries in Japan

1.15. Current Status of Fisheries in Japan

1.16. Current Status of Fisheries in Japan

1.17. Current Status of Fisheries in Japan

1.18. Current Status of Fisheries in Japan

1.19. Current Status of Fisheries in Japan

1.20. Current Status of Fisheries in Japan

1.21. Current Status of Fisheries in Japan

1.22. Current Status of Fisheries in Japan

1.23. Current Status of Fisheries in Japan

1.24. Current Status of Fisheries in Japan

1.25. Current Status of Fisheries in Japan

1.26. Current Status of Fisheries in Japan

1.27. Current Status of Fisheries in Japan

1.28. Current Status of Fisheries in Japan

1.29. Current Status of Fisheries in Japan

1.30. Current Status of Fisheries in Japan

1.31. Current Status of Fisheries in Japan

1.32. Current Status of Fisheries in Japan

1.33. Current Status of Fisheries in Japan

1.34. Current Status of Fisheries in Japan

1.35. Current Status of Fisheries in Japan

1.36. Current Status of Fisheries in Japan

1.37. Current Status of Fisheries in Japan

1.38. Current Status of Fisheries in Japan

1.39. Current Status of Fisheries in Japan

1.40. Current Status of Fisheries in Japan

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The 39th Scientific Symposium of UJNR Aquaculture Panel

October 25th – 26th, 2010

Kagoshima University Inamori Hall, Kagoshima

The Present and Future of the Aquaculture Industry

Aim of the symposium

Based on the goals of the third five-year-plan of FRA that will begin in 2011 and the NOAA and USDA strategic plans for aquaculture, the focus for this meeting will be to present and discuss issues concerning the history and role of aquaculture as well as future production systems and strategies used by each nation to promote aquaculture production, both from the standpoint of the farmer and the consuming public. The primary focus will be on problems faced by the aquaculture industries of the two nations and strategies and solutions to create competitive new and existing aquaculture industries. We will explore current aquaculture systems that are expanding and aquaculture systems that have the potential to expand in the future. We will consider the research needs to maximize the future potential of these industries by examining aquaculture production systems. Aquaculture systems include the whole process from spawning of a certain species to consuming the product including broodstock/hatchery, cultivation, harvest, processing and distribution as a commodity. From this exchange we expect to highlight key bottlenecks that need to be addressed in future meetings. In particular, we will propose a direction for our aquaculture industries and aquaculture research areas that are useful and necessary to promote effective consumer friendly industries that support our seafood culture and consumption. We will present and discuss issues about the history, role, and processes from producing to consuming, process of making a profit and how aquaculture can meet consumer needs.

Program

Monday, October 25th, 2010 Registration 11:30 – 14:00

Opening remarks (Takaji Iida, Japan Panel Chair) 13:00 – 13:10

Welcome greeting (Tadahide Noro, Dean of the Faculty of Fisheries) 13:10 – 13:20

Aim of the symposium (Makoto Yamasaki, Deputy Secretary General) 13:20 – 13:25

Session I. Overview of Aquaculture Research Policy

(Moderators: Walton Dickhoff, Hisashi Yokoyama)

1. Course of the Research for Sustainable Aquaculture in Japan
Fuminari Ito (National Research Institute of Aquaculture, Fisheries
Research Agency) 13:30 – 13:48
2. Aquaculture Research Priorities and Opportunities 2010-2015 and beyond:
a USDA and Interagency Perspective
Jeffrey Silverstein (USDA, Agricultural Research Service) 13:48 – 14:06
3. Current Policy of Aquaculture Industry and Research
Mitsuyuki Hirai (Fisheries Agency, Ministry of Agriculture, Forestry and
Fisheries) 14:06 – 14:24

4. Aquaculture Policy and Research Priorities from the NOAA Perspective
Robert N. Iwamoto (NOAA, Northwest Fisheries Science Center) 14:24 – 14:42

Break 14:42 – 15:02

Session II. Aquaculture Industry Overview and Research Planning

(Moderators: Marty Riche, Takashi Iwasaki)

5. Aquaculture in North America: Present Status and Future Opportunities
Paul G. Olin (UCSD, Scripps Institution of Oceanography) 15:02 – 15:20
6. Overcoming Technical Barriers to the Sustainable Development of
Competitive Marine Aquaculture in the United States
John A. Hargreaves (Aquaculture Assessments LLC) 15:20 – 15:38

Session III. Techniques of Aquaculture Production

(Moderators: James Sullivan, Susumu Uji)

7. A. Challenge to Technology Development of the Year Round Egg Production
in Kan-pachi Amberjack *Seriola dumerili*: Advanced Spawning Technique
Kazuhiisa Hamada (National Research Institute of Aquaculture, FRA)
..... 15:38 – 15:56
8. Spawning and Larval Rearing of Yellowtail Amberjack (*Seriola lalandi*) in
Southern California
Kevin Stuart (Hubbs-SeaWorld Research Institute) 15:56 – 16:14

Break 16:14 – 16:34

(Moderators: Robert Iwamoto, Akiyuki Ozaki)

9. The Development of Aquaculture Techniques for Greater Amberjack
(*Seriola dumerili*) Using Artificially Produced Seed : Technological
Advancement of the Seed Production
Hiroshi Hashimoto (Shibushi Station, National Center for Stock
Enhancement, FRA) 16:34 – 16:52
10. Success of Artificial Completion of the Japanese Eel (*Anguilla japonica*)
Life Cycle
Hiroshi Imaizumi (Shibushi Station, National Center for Stock
Enhancement, FRA) 16:52 – 17:10
11. Offshore Mussel Farming in Southern New England; Recent Results
Scott Lindell (Marine Biological Laboratory, Woods Hole) 17:10 – 17:28

Announcement 17:28

Tuesday, October 26th, 2010

Registration 9:00 – 9:30

Session IV. Management, Social and Economic Issues of the Aquaculture Industry

(Moderators: Paul Olin, Fuminari Ito)

12. Structural Problem of Fish Farming Industry in Japan
Masaaki Sano (Faculty of Fisheries, Kagoshima University) 9:20 – 9:38

13. The Political Economics of United States Aquaculture
Gunnar Knapp (Institute of Social Economic Research, University of
Alaska) 9:38 – 9:56

Session III. Techniques of Aquaculture Production; continued

(Moderators: Jeffrey Silverstein, Mitsuyuki Hirai)

14. Fish Nutrition and Fish Health – Recent Progress in Japan –
Shunsuke Koshio (Faculty of Fisheries, Kagoshima University) 9:56 – 10:14
15. The Future of Aquaculture Feeds in the United States
Michael B. Rust (Resource Enhancement and Utilization Technologies
Division, Northwest Fisheries Science Center) 10:14 – 10:32

Break 10:32 – 10:52

(Moderators: Cheng-Sheng Lee, Tetsuro Shibuno)

16. Progress of DNA Marker-Assisted Breeding in Mariculture Finfish
Akiyuki Ozaki (National Research Institute of Aquaculture, FRA) 10:52 – 11:10
17. Biotechnology for Competitive Aquaculture
Walton W. Dickhoff (Resource Enhancement and Utilization Technologies
Division, Northwest Fisheries Science Center) 11:10 – 11:28
18. Development and Evaluation of Real-time Loop-Mediated Isothermal
Amplification Methods for the Rapid Detection of Penaeid Viruses
Tohru Mekata (National Research Institute of Aquaculture, FRA) 11:28 – 11:46

Lunch Break 11:50 – 14:00

————— Poster Session —————

Session V. Supporting Techniques of Aquaculture

(Moderators: Scott Lindel, Shusaku Kadowaki)

19. Bioindicator and Biofilter Function of *Ulva* spp. in Fish Farm Environments
Hisashi Yokoyama and Yuka Ishihi (National Research Institute of
Aquaculture, FRA) 14:15 – 14:33
20. Inland Marine Fish Culture in Low-Salinity Recirculating Aquaculture
Systems
Marty A. Riche (U.S. Dept. of Agriculture, Agricultural Research Service)
..... 14:33 – 14:51
21. Landbased Poly-Eco-Aquaculture of Abalone and Seaweed in the Small
Scale Recirculating System Using the Recycled Frozen Container
Mohammad M. Rahman (Faculty of Fisheries, Kagoshima University)
..... 14:51 – 15:09

Break 15:09 – 15:24

(Moderators: Kevin Stuart, Makoto Yamasaki)

22. Techniques for Current Control in Tank; Lessons Learned from Rearing
Larvae of Red Spotted Grouper *Epinephelus akaara*

Takashi Iwasaki (National Research Institute of Aquaculture, FRA) ...	15:24 - 15:42
23. Pond-to-Plate Analysis of the US Farm-Raised Catfish Industry	
Terrill R. Hanson (Department of Fisheries and Allied Aquacultures, Auburn University)	15:42 - 16:00
24. Beneficial Meat Quality Induced by Diets in Yellowtail	
Itaru Shioya (Central research laboratory, Nippon Suisan Kaisha Ltd.)	16:00 - 16:18
Break	16:18 - 16:33

Session VII. Stock Enhancement

(Moderators: Gunnar Knapp, Tohru Mekata)

25. A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage Conditioning for Stocking Japanese Flounder, <i>Paralichthys olivaceus</i> , in Wakasa Bay, Japan	
Michelle L. Walsh (University of New Hampshire)	16:33 - 16:51
26. Acoustic Conditioning and Ranching of Black Sea Bass <i>Centropristis striata</i>	
Scott Lindell (Marine Biological Laboratory, Marine Resources Center, Woods Hole)	16:51 - 17:09
Closing remarks (Michael B. Rust, U.S.A. Panel Chair)	17:09 -
Announcement	- 17:20

Satellite Symposium in Shibushi

29th October 10:30 –

At a meeting room in Hotel Volver A Daguri

Hatchery, the Present State and Issues

(Moderator: Fuminari Ito)

1. Artificial Completion of the Japanese Eel, *Anguilla japonica*, Life Cycle: Challenge to Mass Production
Yoshitsugu Masuda (Shibushi Station, National Center for Stock Enhancement, FRA)
2. Spawning and Larval Rearing of Yellowtail Amberjack (*Seriola lalandi*) in Southern California 2
Kevin Stuart (Hubbs-SeaWorld Research Institute)
3. A Challenge to Technology Development of the Year Round Egg Production in Kan-pachi Amberjack *Seriola dumerili*: Proposal of New Systematized Aquaculture
Kazuhisa Hamada (National Research Institute of Aquaculture, FRA)
4. Sablefish Hatchery Methods. An Overview of Hatchery Design, Brood Stock Spawning, and Egg and Yolk Sac Larvae Incubation Procedures at the Manchester Research Station
Michael B. Rust (Resource Enhancement and Utilization Technologies Division, Northwest Fisheries Science Center)
5. Development of A Visualizing Method for Physical Injury to Fish Larvae
Susumu Uji (National Research Institute of Aquaculture, FRA)

Course of the Research for Sustainable Aquaculture in Japan

Fuminari ITO*

Abstract : World aquaculture has grown dramatically in the last 50 years, mainly in Asia, and is expected to increase further. In the meantime, aquaculture production of Japan has stabilized and occupies an important position in the entire fishery, but the effects of that situation in Japan are severe. The situation is due to increased costs of fuel oil, fish meal for feed, the high cost of quality fish for stocking and sluggish fish prices caused by the economic recession, along with losses caused by red tide and outbreaks of disease. Due to those factors, the aquaculture industry in Japan is faced with high production costs and low prices for its products.

To improve the management of aquaculture, it is necessary to shift to a sustainable and profitable structure through a review of the entire production process. We organized an overview of aquaculture production systems, and have discussed research problems and directions for solving them. To achieve sustainable aquaculture, the industry must reduce the environmental impacts, as well as need to be profitable. For low impact on the environment, research and technological innovation are needed on larvae and fry production that is independent on natural resources, improvement of culture methods and feed quality and so forth. To increase revenue, it is necessary to practice continual cost savings by analyzing each process of associated with aquaculture production and development of a way to add value to products. Thus, it is important that aquaculture becomes regarded as an integrated production system.

Keywords : sustainable aquaculture, profitability, aquaculture production systems

Production of aquaculture in the world has grown dramatically, and the increased rate since 1970 is much higher than that of world population growth during the same period according to the Food and Agriculture Organization (FAO) as can be found in The State of World Fisheries and Aquaculture (2008)¹⁾. Recently, aquaculture has been considered to be the answer for food security since the depletion of natural fisheries resources is concerned. While the aquaculture industry overall continues to grow, its status in Japan is not necessarily favorable. However, considering global food supply and food security, it is said that future continued expansion of the industry is strong. This paper introduces the current state of aquaculture in the world and Japan,

indicating the direction of research is heading in the Fisheries Research Agency.

Situation of aquaculture in the world

The global aquaculture industry continues unprecedented development. Over the past 20 years aquaculture production has increased rapidly, while capture fisheries production has not (Fig. 1). Aquaculture accounted for 47 percent of the global supply of edible fish and shellfish in 2006. Of that, 90 percent is produced in Asia (FAO Statistics, 2006). This dominance is mainly due to China's enormous production, most of which consists of freshwater fish. Looking at the top ten countries of aquaculture

production, excluding seaweeds, Asian countries dominate, with Japan eighth on the list. Chile and

Norway, the world's leading producers of salmon, occupy the positions of seventh and ninth.

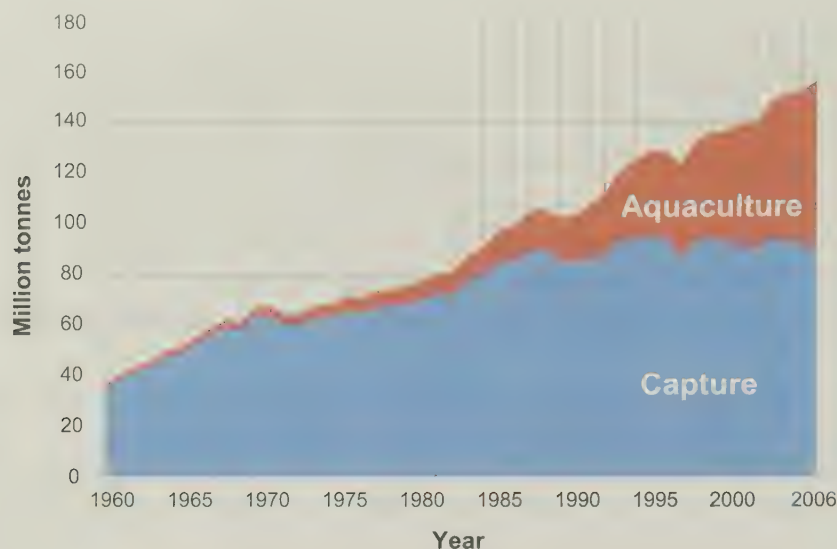


Fig. 1. Capture and aquaculture production in the world (The state of fisheries and aquaculture 2008¹⁾)

The aquaculture situation in Japan

Aquaculture production in Japan in 2008 was 1.19 million tons, with a value of 4,790 billion yen, accounting for 21% of domestic fisheries production.

Since the late 1980s, in the face of falling capture fisheries production, aquaculture production has been stable, and has increased its presence accounts for a relatively large proportion in the fishery (Fig. 2). However, Japan's aquaculture industry is in poor

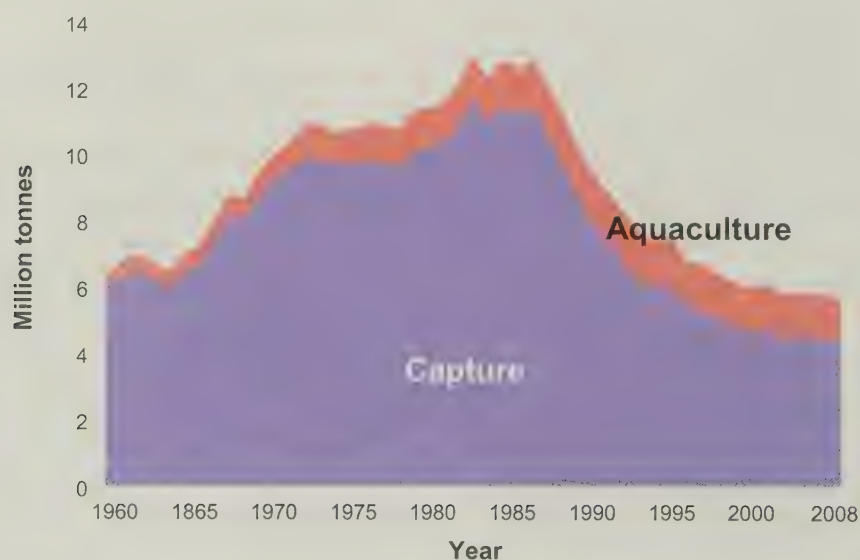


Fig. 2. Capture and aquaculture production in Japan (from the statistics of fisheries and aquaculture 2008²⁾)

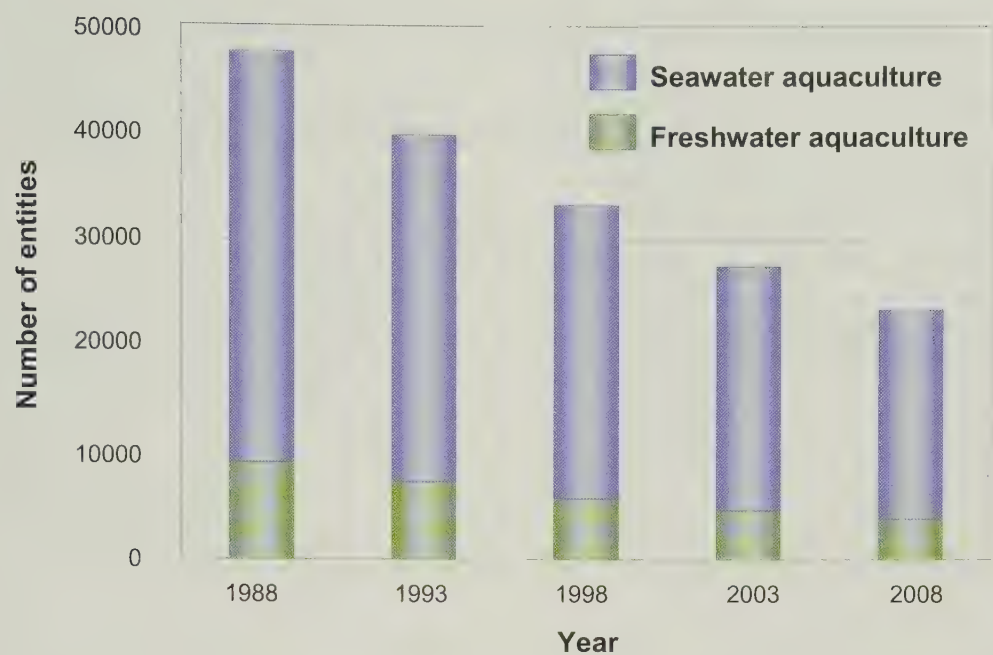


Fig. 3. Number of management entities in Japan (from the statistics of fisheries and aquaculture 2008²⁾)

shape because of increased production costs due to rising prices of fuel oil and fish meal, restrained purchasing of high quality fish and a decline of fish prices due to business recession. Other factors have been outbreaks of red tides and fish diseases. These circumstances have engendered the production structure with high production costs and low prices, and have given rise to problems such as a decrease in the number of management entities (Fig. 3), an aging work force and lack of successors.

In contrast, some new industries, as bluefin tuna aquaculture, have been developed in Japan. Aquaculture production of tuna is estimated to have reached 10,000 tons in 2010, up from less than 500 tons in 1999. The number of management entities entering tuna aquaculture has increased. That development still does not supply enough bluefin tuna to satisfy domestic demand. That is due to reduced production in world capture and aquaculture in the Mediterranean and by the regulation of tuna fisheries due to heightened international interest in the conservation of the resource.

In addition, hatchery production is one of the

Table 1. Number of production species in Japan (from seed production for stock enhancement, acquisition / plantation record 2007³⁾)

	Aquaculture	Plant
Fish	25	39
Crustacean	3	11
Shellfish	19	24
Others	4	7

(except for salmonids, eel and algae)

technologies for which the Japanese are proud. Table 1 shows hatchery production of various species in Japan. Many species are produced both for aquaculture and stock enhancement³⁾. Further, consumers in Japan ask for an abundance of seafoods that are rich in variety. Therefore, we think that Japanese aquaculture has the potential for further development.

Problems associated with sustainable aquaculture

Although the global aquaculture industry has made remarkable progress, it cannot be considered

that aquaculture will continue to grow within the level of current technology because fish aquaculture can result in pollution of the environment and the amount of feed available to be used in it is limited. Then, as mentioned above, the present situation with respect to the aquaculture industry in Japan is in a critical condition. A new perspective is needed for further development of the industry. Sustainability is an important consideration for regeneration of profitable aquaculture since industries cannot carry on if they operate at a loss. Research must be focused on not only sustainability but also profitability. It is necessary to first analyze the entire aquaculture enterprise. Fig. 4 shows an overview of aquaculture systems that was organized for digging out the problems. We are apt to think that only fish and shellfish production processes make up an aquaculture system. However, production needs support sections such as facilities and process management, along with post-harvest activities such as processing and distribution. Furthermore, management cannot be neglected, since aquaculture

is an economic activity. We have tried to distill problems from each of these categories through surveying the overall aquaculture system.

Research contents for sustainability and profitability are shown in Tables 2 and 3. In sustainability, issues including environmental improvement, appropriate amount of feeding, reduction of fish meal in feed, integrated multi-trophic aquaculture that combines species from different nutritional levels in the same system, and systems that include production of eggs spawned by fish reared from artificial fertilization, need to be addressed in order to reduce impacts on the environment. Complete aquaculture reduces the impact of fishing on native resources. Worker safety and reduction of labor are taken up for improvement of the work environment. Furthermore, mechanization, land-based aquaculture and offshore aquaculture require modernization. For profitability, it is important for management to realize the characteristics and trends of balance. Marketing is very important issue as well. There are

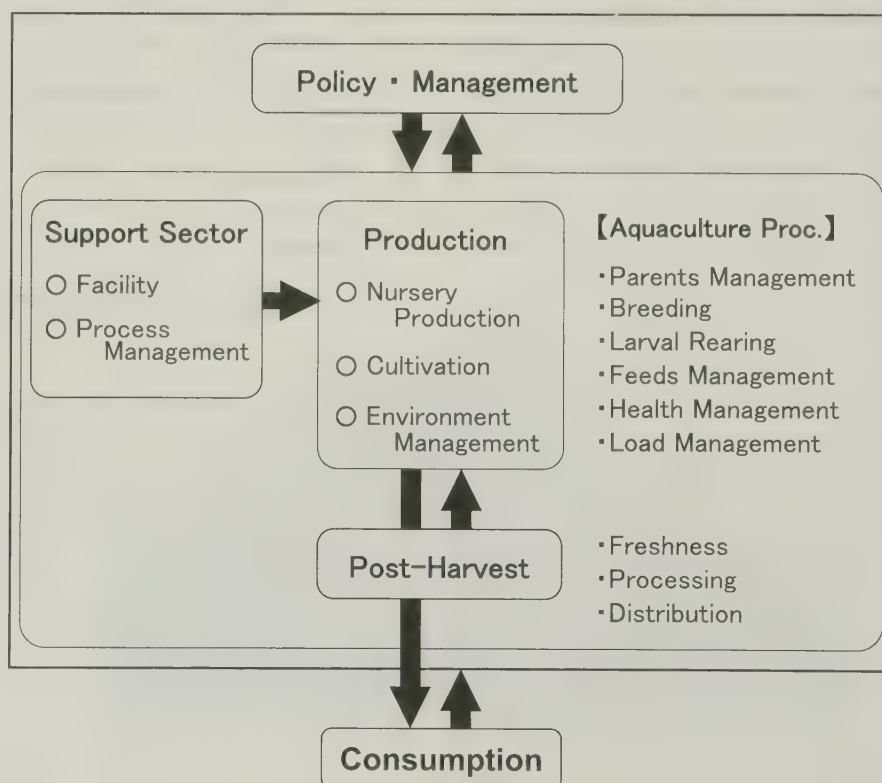


Fig. 4. Illustration of an aquaculture production system (fish aquaculture)

Table 2. Research contents in sustainable aquaculture

Sustainable Aquaculture
◆ <u>Reduce impact on environment</u> • environmental improvement • appropriate amount of feeding • reduction of fish meal in feed • integrated multi-trophic aquaculture • complete aquaculture (reduces the impact of fishing on native resources) ◆ <u>Improvement of the work environment</u> • worker safety • reduction of labor (mechanization) ◆ <u>Modernization</u> • mechanization • land-based aquaculture • offshore aquaculture

many specific problems in productivity improvement and cost savings. Maturation control, stable hatchery production, optimum rearing conditions, breeding, prevention of disease, nutrient enrichment of live feed organisms, and others need to be considered. Integrated multi-trophic aquaculture is important not only to reduce impacts on the environment but also for productivity improvement and cost savings. This practice is new and has attracted considerable attention since it promotes economic and environmental sustainability. Researches on food safety, quality control and product traceability are needed to add value to aquaculture products. Future research needs to take into account aquaculture as an integrated production system, recognize the relative importance of each of specific issue and how each affects the overall aquaculture system.

Future research with the aim of profitability and sustainability

This paper indicates that aquaculture has to

Table 3. Research contents in profitable aquaculture

Profitable Aquaculture
◆ <u>Management</u> • realize characteristics and trends of balance • marketing ◆ <u>Productivity improvement and cost savings</u> • maturation control • stable hatchery production • optimum rearing conditions • breeding (tolerance to disease, high growth rate, etc.) • prevention of disease • nutrient enrichment of live feed organisms • integrated multi-trophic aquaculture ◆ <u>Adding value</u> • food safety • quality control • product traceability

incorporate integrated production systems from production to distribution and consumption. We should address aquaculture research that includes management for producing a profit to post-harvest for selling the products at high prices, as well as production technology for improving productivity. The Fisheries Research Agency aims to help develop a sustainable aquaculture industry through research and technological innovation.

References

- 1) The state of world fisheries and aquaculture, 2008: Food and Agriculture Organization of the United Nations.
- 2) The statistics of fisheries and aquaculture, 2008: Statistical Yearbook of Ministry, Forestry and Fisheries.
- 3) Seed production for stock enhancement, acquisition / plantation record, 2007: National Center for Stock Enhancement, Fisheries Research Agency.

Aquaculture in the United States of America: Present Status and Future Opportunities

Paul G. OLIN*

Abstract : This represents a review of the status and trends in aquaculture development in the United States. The paper covers current levels of American production and species diversity, production trends over the last decade, available resources and governmental support, and projections for future industry growth and development.

Total aquaculture production in the United States of 496,907 metric tonnes in 2008 generated US\$924 million. The aquaculture industry grew between 1998 and 2008, increasing 12 percent in tonnage and 19 percent in value. This increase resulted entirely from the shellfish sector, where production increased 65 percent from 127,059 to 209,775 tonnes, with a corresponding 186 percent increase in value from US\$113 to US\$323 million. In contrast, the finfish sector declined 9 percent in both tonnage and value with 2008 production of 287,132 tonnes worth US\$601 million.

The increase in shellfish production was achieved primarily through additional production of Pacific and Eastern oysters, hard clams, manila clams and red swamp crayfish. While still the dominant species, the U.S. channel catfish industry has experienced strong competition as a result of significant increases in imports of basa and tra from Southeast Asia and has declined in recent years. In 2008, American catfish growers produced 201,000 metric tonnes of fish valued at US\$389 million.

FAO has projected the need for an additional 40 million metric tonnes of seafood by 2030 to meet anticipated increases in global demand. There is potential for significant increases in production in the United States. The NOAA Aquaculture Program within the National Marine Fisheries Service released a Ten Year Plan for Marine Aquaculture in 2007, which identified opportunities for potential growth in the sector. While not specific targets, estimates are that domestic aquaculture production has the potential to increase in value from US\$1 billion to more than US\$3 billion by 2025. This additional production would be comprised of 760,000 tons of finfish, of which 590,000 tons would be marine finfish, 47,000 tons of crustaceans, and 245,000 tonnes of molluscs.

Future significant growth in the American aquaculture industry will likely follow the successful model demonstrated by the Atlantic salmon industry in Canada with new technologies enabling net-pen culture to move further offshore. There are ample areas for this expansion and pilot projects in the U.S. have demonstrated the viability of this approach. However, in some regions there is considerable opposition and whether a significant industry sector develops will require the establishment of a regulatory regime that insures environmental protection while enabling the economic viability of aquaculture ventures.

Key Words : aquaculture, United States, shellfish, finfish, production

The total aquaculture production in the United States of 496,907 tonnes in 2008 generated US\$924 million (FAO FishStat Plus, 2010). The aquaculture industry grew between 1998 and 2008, increasing 12% in tonnage and 19% in value. This increase resulted entirely from the shellfish sector, where production increased 65% from 127,059 to 209,775 tonnes, with a corresponding 186% increase in value from US\$113 to US\$323 million (Figure 1). In contrast, the finfish sector declined 9% in both tonnage and value, with 2008 production of 287,132 tonnes valued at US\$601 million (Figure 2).

The aquaculture industry in the United States continues to be dominated by production of channel catfish (*Ictalurus punctatus*) that generated US\$390 million in 2008 from sales of 233,564 tonnes. That represented 42 percent of the total aquaculture product value and 47% of total tonnage (Figures 3 and 4).

Channel catfish farming began in the 1960s in the southeastern states of Louisiana, Arkansas, Alabama, and Mississippi, which combined account for more than 90% of domestic catfish production. The development of feed mills and processing capacity enabled significant expansion of the industry and from 1982 to 2002 the pond area approximately

doubled and production increased six-fold. Since then, production has declined substantially as the industry has faced intense competition from high quality *Pangasius* fillets imported from Asia and escalating feed prices as corn is diverted for ethanol production. Imports of frozen *Pangasius* fillets now account for 50% of the catfish fillet products sold. Catfish feed prices rose 34% between 2007 and 2008, rising from US\$289 to US\$388/tonne.

Despite these factors, highly efficient catfish farmers remain competitive. Between 2002 and 2008, the catfish industry in the four major producing states lost 23,593 ha of water surface area. This represents an average water surface area decline of 36%; however, average production declined by only 4% (Table 1). The average price of catfish was US\$1.69/kg in 2007 and rose slightly to US\$1.71/kg in 2008.

Atlantic salmon and rainbow trout contribute significantly to the finfish aquaculture sector in the US with production in 2008 of just over 16,000 tonnes each, valued at US\$45 million and US\$50 million (Figures 3 and 4). Rainbow trout are produced primarily in raceways in Idaho which accounts for about 50% of production. Other major trout producing states include California, North Carolina,

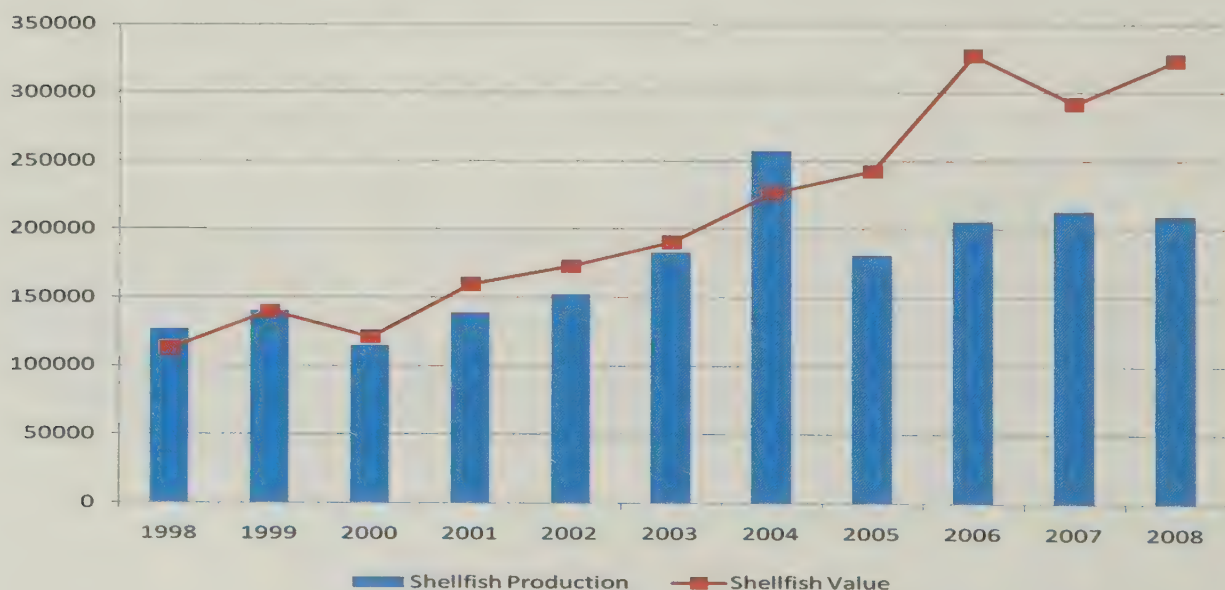


Fig. 1. United States shellfish production (tonnes) and value (US\$1,000), 1998-2008 (FAO FishStat Plus 2010).

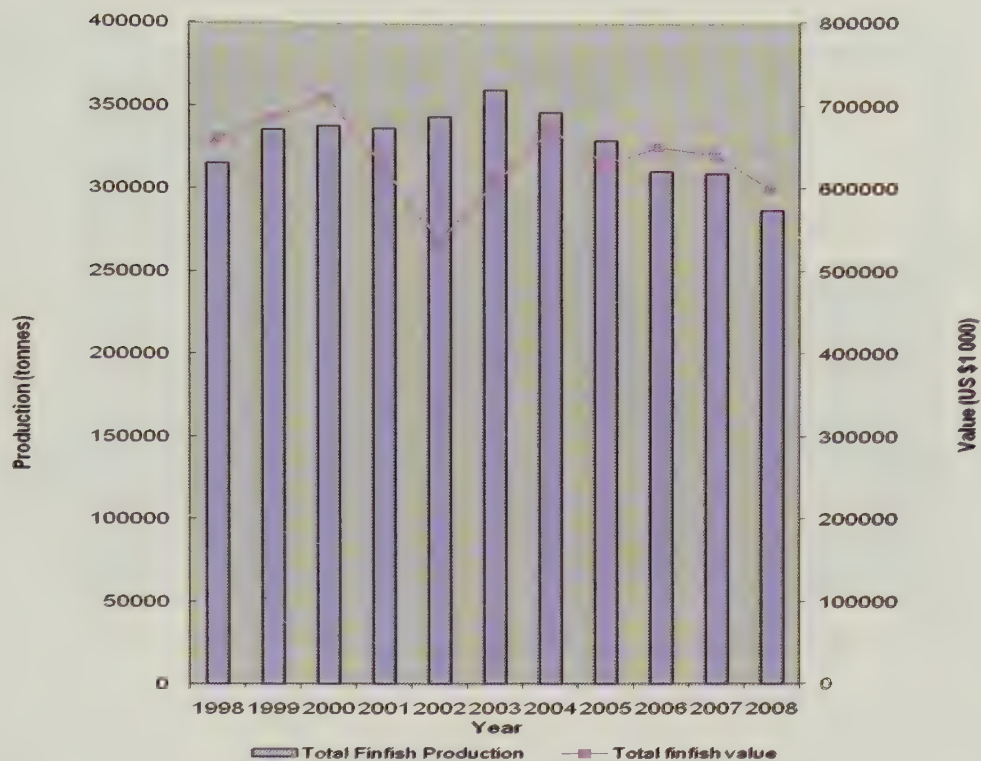


Fig. 2. United States finfish production (tonnes) and value (US\$1,000), 1998-2008 (FAO FishStat Plus 2010).

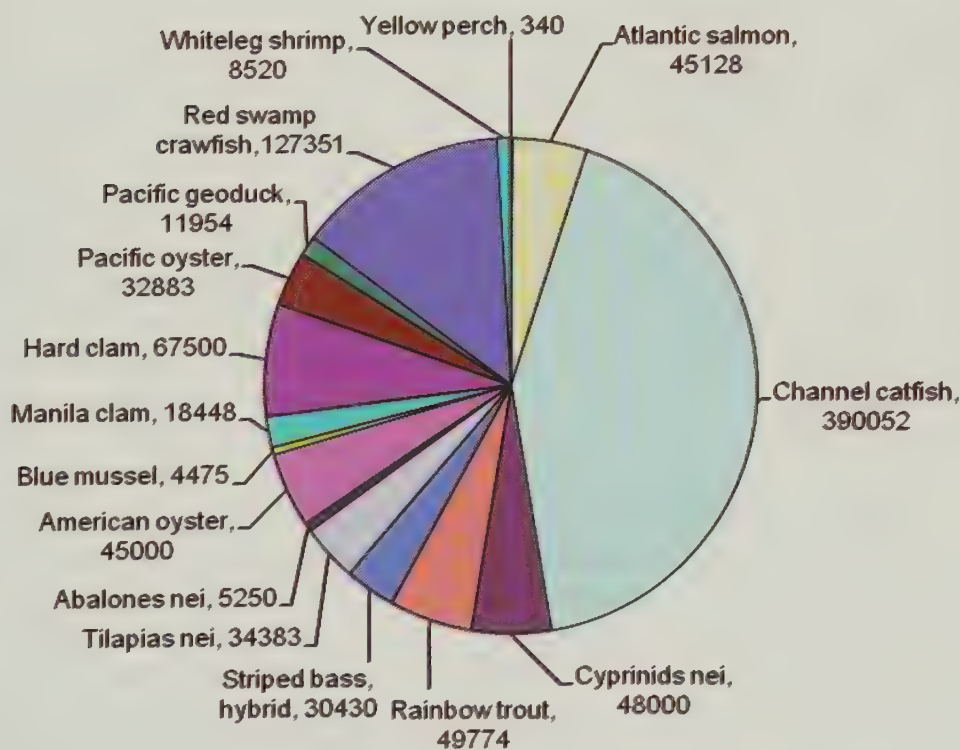


Fig. 3. United States Aquaculture Market Values in 2008, in US\$1,000 (FAO FishStat Plus, 2010).

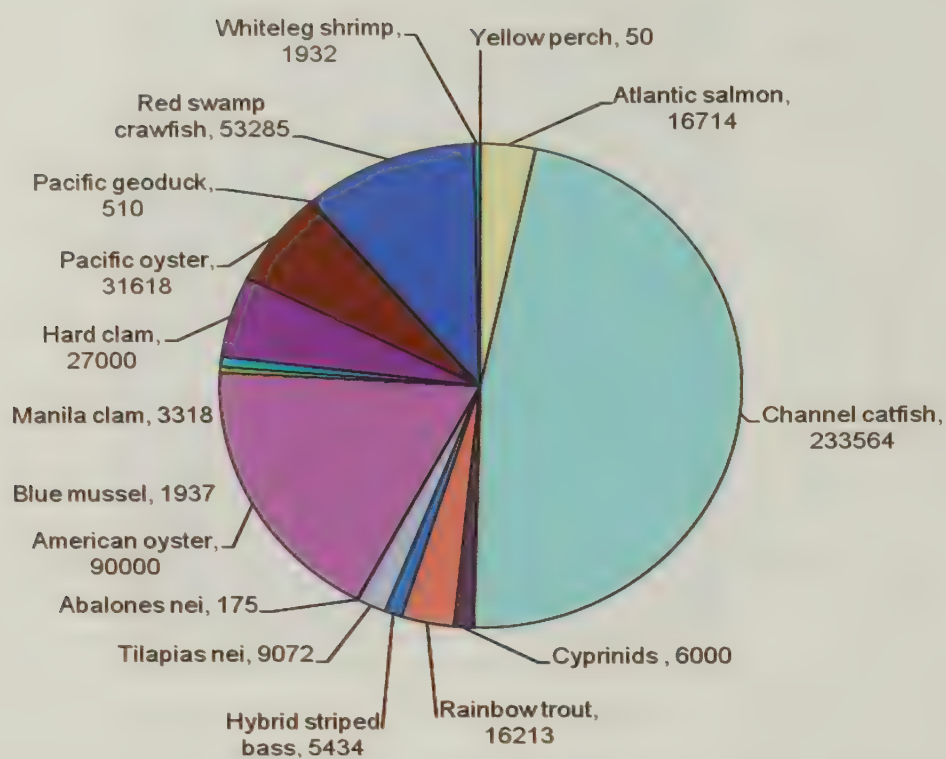


Fig. 4. United States Aquaculture Production 2008 in tonnes (FAO FishStat Plus, 2010).

Table 1. Catfish farm water surface area decline and production by major states

State	Lost production Area (ha)	Percent decline in area	Production 2002 (US\$1,000)	Production 2008 (US\$1,000)	Percent change in production
Alabama	2,226	22	76,045	93,254	23
Arkansas	5,261	36	56,380	64,263	14
Louisiana	13,152	29	15,812	11,883	-25
Mississippi	2,954	55	243,226	206,288	-15
	23,593*	36**	391,463*	375,688*	-4

Source: USDA, NASS (2009).

* represents total value for four states

** represents average value for four states

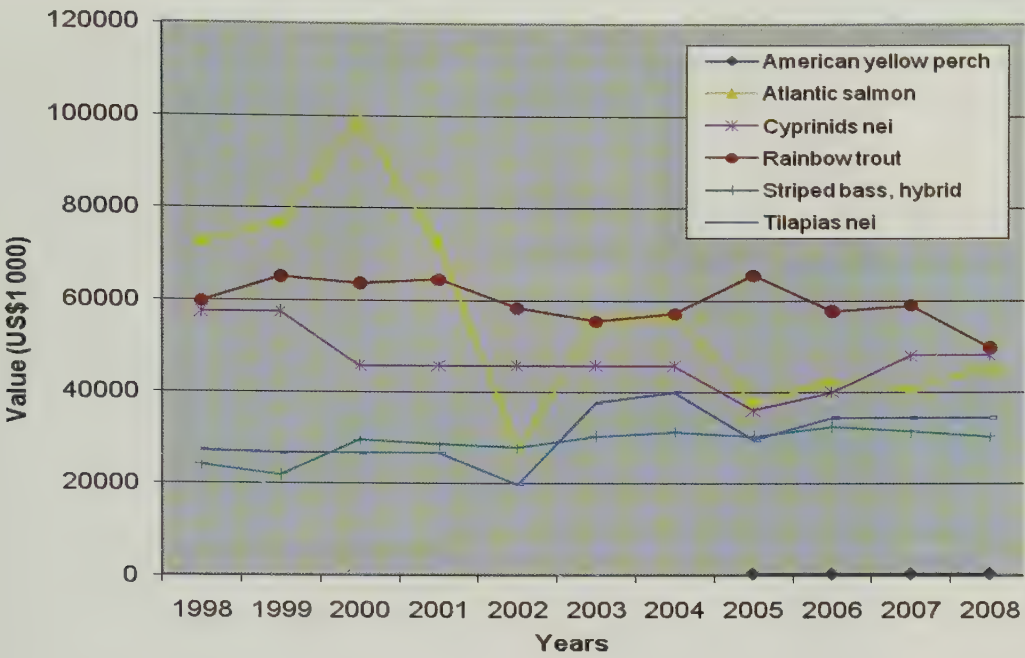


Fig. 5. United States finfish value trend 1998-2008 in US\$1,000 (FAO FishStat Plus, 2010). Excludes channel catfish.

and Pennsylvania. Atlantic salmon are produced in ocean net-pens off the coast of Maine and in Puget Sound, Washington. Other farmed finfish include cyprinids, hybrid striped bass, tilapia, and yellow perch, a newly emerging species. Production of these species has been stable or declining from 1998 to 2008 (Figure 5).

Shellfish are cultured in every coastal state but production centers are located primarily along the coasts of the northeast, the Gulf of Mexico, and the Pacific northwest. Principal cultured species are Atlantic and Pacific oysters, blue mussels, hard clams, crawfish, abalone, and Geoduck clams. Production of crawfish, hard clams, Atlantic oysters and Pacific oysters comprise 84% of shellfish value worth US\$127, 68, 45, and 33 million, respectively. In contrast to the stable or declining trend observed in finfish production, all shellfish species increased in production and value from 1998 to 2008 (Figure 6).

FAO has projected the need for an additional 40 million tonnes of seafood by 2030 to meet anticipated increases in global demand (FAO, 2009). The United States is projected to require 1.1 million additional tonnes of seafood by 2030 to maintain current levels

of consumption, and health advisories recommend that Americans double their seafood consumption. Domestic seafood production through fisheries and aquaculture falls far short of supplying current domestic needs and currently about 85% of the seafood consumed is imported. This creates a US\$10 billion annual seafood trade deficit. Approximately 50% of imported seafood results from aquaculture production, primarily in Asia. The United States Department of Commerce (DOC) National Oceanic and Atmospheric Administration (NOAA) recognizes the need to expand aquaculture production to meet future seafood needs and reduce the seafood trade deficit. The NOAA National Marine Fisheries Service Aquaculture Program has a Ten Year Plan in which the possibility for significant increases in aquaculture production are described (NOAA, 2007). This additional production would be comprised of 760,000 tonnes of finfish, of which 590,000 tonnes would be marine finfish, 47,000 tonnes of crustaceans, and a 245,000 tonne increase in mollusc production.

Growth in the marine finfish sector will require technologies that enable aquaculture in offshore environments given environmental and user



Fig. 6. United States shellfish value trend from 1998-2008 (US\$1,000).

group conflicts in nearshore areas. Of paramount importance is the need to develop hatchery technology for a number of new species including southern flounder, red drum, Atlantic cod, halibut, yellowtail, cobia, yellowfin tuna, and pompano. In

particular, cobia is viewed as a leading candidate for significant expansion of ocean net pen culture with the development of fingerling production capacity and extraordinary growth rates to 6 kg in one year. One pilot scale facility cultured cobia near Puerto

Rico, and two facilities are using ocean net pens in Hawaiian waters, raising Hawaiian amberjack and moi. The University of New Hampshire's Atlantic Marine Aquaculture Center has piloted culture of Atlantic cod, halibut, and flounder. There is also a project supported by the Hubbs SeaWorld Research Institute to develop an ocean cage production operation in Mexico raising Pacific yellowtail, striped bass, and white seabass.

To date, there has been no significant private investment in offshore aquaculture, largely because of regulatory uncertainties and opposition by groups fearful of adverse environmental impacts, competition, and user group conflicts. The DOC and NOAA recognize this and in February 2011 released draft aquaculture policies designed to provide guidance for the development of a comprehensive regulatory framework to enable aquaculture development in the U.S. The policy has eight priority policy statements that are designed to "Create a business climate and technological base for industry to develop sustainable aquaculture" (DOC, 2011). The companion policy of NOAA contains nine policy statements to "Enable sustainable aquaculture that provides domestic jobs, products, and services and that is in harmony with healthy, productive, and resilient marine ecosystems, compatible with other uses of the marine environment, and consistent with the National Policy for the Stewardship of the Ocean, our Coasts, and the Great Lakes" (NOAA, 2011).

These are promising policies that provide a foundation for the development of a regulatory framework for aquaculture. Pilot projects in the U.S. have demonstrated the viability of net pen farming of marine finfish. However, in some regions there is considerable opposition and whether a

significant industry sector develops will depend on the establishment of a regulatory regime that insures environmental protection while enabling the economic viability of aquaculture ventures.

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Spawning and Larval Rearing of California Yellowtail (*Seriola lalandi*) in Southern California

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Abstract : Hubbs-SeaWorld Research Institute (HSWRI) has been rearing California yellowtail (*Seriola lalandi*) in San Diego, California, since 2003. The broodstock spawn naturally between 16.5-22.0°C in a 140 m³ maturation pool. Egg quality measurements have shown inconsistent spawn quality, with highest quality seen early in the spawning season. Hatch rates ranged from 10-90% and survival to first feeding at two days post-hatch (dph) ranged from 5 -80%. Recent research results on yellowtail larvae have shown no significant differences in growth or survival at 10 dph among egg stocking densities of 50, 100, and 200 eggs/L. There were no significant differences in growth or survival when larvae were fed at densities of 15, 30, and 45 rotifers/ml. Larvae were able to consume 1st instar brine shrimp (*Artemia franciscana*) as early as 6 dph. Finally, we determined that greenwater rearing conditions created using either INVE SanoLife® ALG, algae paste, or live algae all produced similar results. Current culture protocols for production are based on these results and include 1) stocking eggs at a density of 100/L; 2) maintaining water temperature at 21.0-22.0°C; 3) providing rotifers (*Brachionus rotundiformis*) from 2 to 9 dph at 20/ml; 4) moving larvae from tall, narrow 1600 L egg incubators to shallow, wide 8,000 L tanks at 10 dph; 5) providing 1st instar *A. franciscana* from 6-10 dph and 2nd instar *A. franciscana* from 10-35 dph; 6) 24 hr overhead light at 5,000-13,000 lux; and 7) using Green water from 2-17 dph. These procedures yielded consistent survival rates from egg to juvenile of up to 5%. High larval mortality, from unidentified causes at around 17-20 dph and deformity rates as high as 40% are focal areas for future improvement.

Keywords : *Seriola lalandi*, aquaculture, green water culture, live feed management

Introduction

The California yellowtail, (*Seriola lalandi*; CYT), is a member of the family Carangidae, found in temperate waters of the Pacific and Indian oceans (Baxter, 1960; MacCall *et al.*, 1976; Sumida *et al.*, 1985; Kolkovski and Sakakura, 2007). In the Eastern Pacific, adult and juvenile CYT range from southern Washington to Chile, including the Gulf of California, and are seasonally abundant in southern California. Larval distribution is limited to warmer waters from as far north as Point Conception and south

to Baja California (Sumida *et al.*, 1985). In the Southern California Bight, CYT larvae have been found from April through October (Sumida *et al.*, 1985). Because of its status as a popular sport and commercially-caught fish, the CYT, along with several other species in the genus *Seriola*, are a focus of the growing worldwide aquaculture industry.

Multiple *Seriola* spp. are cultured commercially in several countries around the Pacific (Benetti *et al.*, 2001; Nakada, 2002; Fowler *et al.*, 2003; Verner-Jeffreys *et al.*, 2006). These species include yellowtail, *S. quinqueradiata* (Japan and Taiwan);

amberjack, *S. dumerili* (Japan and Hawaii); and almaco jack, *S. rivoliana* (Hawaii). CYT is being commercially cultured in Japan, South Australia, and New Zealand, with Japanese farmers relying on wild caught juveniles (Nakada, 2002), and farmers in Australia and New Zealand producing juveniles from domestic broodstock (Fowler *et al.*, 2003). Currently, commercial culture of *Seriola* spp., in the U.S. occurs only in Hawaii (Verner-Jeffreys *et al.*, 2006). However, CYT is considered a top candidate for culture in southern California because of its local availability and high market value. The Hubbs-SeaWorld Research Institute (HSWRI) began an experimental CYT breeding program in 2003 with wild locally caught adults. The research program is conducted from HSWRI's research laboratory in San Diego, California.

Materials and Methods

Egg Collection: Broodstock were collected in 2003 and 2004 off San Diego and Catalina Island, California. The broodstock were housed in a 140 m³ fiberglass pool (9.1 m diameter x 2.4 m deep). The fish were exposed to shaded natural light and ambient seawater temperatures of 13-23°C. The seawater for the broodstock pool was recirculated at a rate of 1,135 L/min using an airlift-driven bead filter (0.7 m³ PolyGeyser Bead Filter, Aquaculture System Technologies, New Orleans, LA). The bead filter performed the critical processes of solids capture and biofiltration. Water supplied to the pool by the airlift flowed by gravity from the top of the pool and a central bottom drain into an egg collector, so all eggs were collected during the study period spanning 2009 through 2010. The egg collector measured 1.27 x 1.14 x 0.64 m and contained a 500 µm mesh bag to collect the eggs before the water returned to the filter. Makeup water drawn from Mission Bay was sand-filtered and sterilized with ultraviolet light before being supplied to the pool at a rate of 5-20 L/min. A supply of pure oxygen was available to maintain dissolved oxygen (DO) levels above 7 mg/L (90-100% saturation) during the summer months.

The egg trap was checked daily at 0800, and any eggs found were collected with a fine

mesh aquarium net. Only the floating eggs were considered viable. Eggs were disinfected with 100 ppm of formalin for one hour prior to stocking. The following egg and larval parameters were recorded: egg diameter, oil diameter, hatch rate (%) and survival to first feeding (%). A picture was taken of a subsample of eggs from the floating portion of the spawning event (Image Pro Plus; Leica MZ16 Macro). Once the picture was taken, the egg and oil diameters were measured from 20 qualifying eggs. A qualifying egg was one that was in full view and could be measured. Hatch rates were determined by setting up ten floating eggs on the day of collection (minus 2 days post hatch [dph]) in each of 10, 1.0 L beakers filled with 800 ml of sterilized seawater. The number of hatched larvae was counted in each beaker, and percent hatch was calculated as the average for the 10 replicates (totaling 100 eggs) for that spawning event. Survival to first feeding tests were conducted using the same methodology as the hatching test, except 0 dph larvae were used instead of eggs. Each day the number of surviving larvae in each beaker was counted until first feeding (2 dph).

Larval Rearing Trials: All larval rearing trials were conducted in the same experimental system, which maintained consistently good water quality. The experimental system consisted of 24,320 L black conical bottom tanks. DO, pH, and temperature were monitored daily using a portable meter (Hatch model HQ40d, Hach Company, Loveland, Colorado). Total ammonia nitrogen, nitrite, and nitrate were measured weekly with Hach test kits (Hach Company, Loveland, Colorado). Separate rearing trials were completed to investigate stocking density (50, 100, 200 eggs/L), rotifer feed density (15, 30, 45 rotifers/ml); brine shrimp weaning (6, 8, 10 dph), and green water replacement (live algae, algae paste, dry algae, SanoLife® ALG (INVE Aquaculture, Inc., Salt Lake City, Utah), clay, tracer dye).

The stocking density was set at 50 eggs/L for all but the stocking density trial. The tanks were supplied with temperature-controlled (22 °C) recirculating seawater at 1.5-2.0 L/min. A containment screen was placed in the center of each tank along with an aeration ring to maintain good water circulation. Three hundred µm screens were used from -2-10 dph and 500 µm screens were used

from 10-14 dph. Fluorescent lights were placed 0.7 m above the tanks for illumination (Lithonia, Conyers, Georgia). The light intensity (1,500 to 3,000 lux) was set at the surface using a light meter (EXTECH Instruments, Waltham, Massachusetts), and light duration was set at 24 hr. The tanks were cleaned once a week for the duration of the trial, and screens were changed as needed.

Live prey was added four times daily at 0700, 1000, 1300, and 1600. Live feed densities were maintained at 15-45 rotifers/mL or 5 *Artemia*/mL throughout each trial. Prey densities were measured twice daily in each tank to maintain the target levels. This was accomplished by taking a 10-20 ml sample of seawater from each tank after which prey counts were made from three, 1 ml aliquots of the total sample. Green water was used for each trial and was developed by adding algae paste (Reed Mariculture, Campbell, California) at a cell level of 300,000-500,000 cells/mL with each feeding. The green water replacement trial was standardized by using similar Secchi disc readings (28-35.6 cm) for the clay (Bentonite Clay, New Mexico Clay Company, Albuquerque, New Mexico) and tracer dye (Bright Dyes, Kingscote Chemicals, Miamisburg, Ohio) treatments.

Larval growth was measured at 0, 3, 10, and 16 dph on subsamples of 50 larvae per treatment. Larvae were euthanized with MS-222 prior to measurement. Twenty larvae were placed under a microscope (Leica MZ16 Macro, Bannockburn, Illinois), and a photo was taken with a digital camera (Image Pro Plus, Media Cybernetics, Bethesda, Maryland). Notochord length (NL) was then measured to the nearest 0.1 mm. All 50 larvae were rinsed with de-ionized water on a 200-600 μ m filter, poured through glass filter paper, and dried on glass filter paper dishes in an 80°C oven for 48 hrs. Dishes were weighed before adding larvae and then after the drying period to the nearest μ g. To calculate the individual dry weight (DW), the difference between the weights was divided by the total number of larvae sampled ($n = 50$). Statistical analyses were performed using Statistica (Statistica, Tulsa, Oklahoma). ANOVA was used to determine differences between treatments, and arcsine transformation of the data was completed when

appropriate.

Larval Production: Eggs were stocked into 1,600 L (1.2 m diameter and 1.6 m deep) cone-bottom larval rearing incubator tanks for culture. Flow rates were maintained at 3-6 turnovers per day, depending on the developmental stage of the larvae. Mild aeration was provided with airstones and bubble ring diffusers. Controlled lighting was installed above the incubator tanks providing an illumination of 7,000-11,000 lux (four 54-watt Metalux® bulbs, Cooper Lighting, Peachtree City, Georgia) at the surface. Larvae were reared in the incubators until 10 dph, at which time they were gently moved to a large diameter (3.6 m), shallow (0.9 m), flat-bottom 6,000 L pool. The seawater supplied to the pools was maintained at 21-22°C. Larvae were reared on recirculating water through metamorphosis (35 dph). The recirculating system was equipped with a fluidized bed, bead filter, and 50 μ m bags to filter any particulates and/or uneaten rotifers or *Artemia*. At approximately 2-3 dph, larvae were fed rotifers (*Brachionus plicatilis*) enriched with Arti-Kol (Nutra-Kol Nutrition Solutions, Western Australia). In order to provide the larvae with enriched rotifers throughout the day, rotifers were cold-stored the day before at 8-10°C in an insulated cooler. The larvae from 2-10 dph were fed rotifers four times per day, maintaining a tank density of 20 rotifers/ml with algae paste (Reed Mariculture, Campbell, California) added to the larval tank with each feeding to achieve a concentration of approximately 300,000-500,000 cells/ml until the larvae were 17 dph. Unenriched 1st instar *Artemia* were fed from 6-14 dph and 2nd instar *Artemia* enriched with Arti-Kol were fed from 14-35 dph. A dry micro diet (Otohime, Reed Mariculture, Campbell, California) was fed starting at 15 dph and overlapped with 2nd instar *Artemia* through to 35 dph.

Results

Egg Production: Each year, spawning occurred when water temperatures were 16-22°C, with egg production decreasing at the end of the season when water temperature was above 21°C. Eggs were found as early as 1600 and as late as 0100, and spawning occurred earlier in the evening as the

Table 1. Egg production and quality numbers for CYT broodstock

Parameter	2009	2010
Number of spawn events	37	43
Egg diameter (mm)	1.36 ± 0.03^b	1.40 ± 0.05^a
Oil diameter (mm)	0.29 ± 0.02	0.31 ± 0.05
Hatch Rate (%)	75 ± 11	70 ± 19
Survival to First Feeding (%)	64 ± 18	63 ± 22

*Values sharing superscripts are not significantly different.

spawning season progressed. Courtship behavior was usually observed early in the afternoon, with pairs of fish swimming faster than normal and a male nudging at the abdomen of the female. The mean egg diameter in 2010 was significantly larger than in 2009; oil diameters also increased but not significantly. Hatch rates and survival to first feeding remained constant from 2009 through 2010 (Table 1).

Larval Rearing Trials: Water quality was similar for all experiments and within acceptable ranges for fish larvae. Water temperature was maintained at $21.4 \pm 0.3^\circ\text{C}$ and salinity was 34.5 ± 0.5 ppt. Dissolved oxygen ranged from 8.0-8.6 mg/L (94-96% saturation), and mean total ammonia nitrogen and unionized ammonia levels were <0.01 and 0.0025 mg/L.

Egg stocking density trials showed no significant differences in growth or survival at 50, 100, and 200 egg/L up to 10 dph. The highest survival ($15.3 \pm 8.1\%$) and the largest larvae (215 ± 41 μg) were produced in the 100 eggs/L treatment, although they were not significantly different from the other treatments.

Larvae were fed 15, 30, and 45 rotifers/ml all showed similar in growth and survival rates, although the largest larvae came from the 45 rotifer/ml treatment. Larvae fed 1st instar *Artemia* at 6 dph had significantly higher growth than larvae fed 1st instar *Artemia* at 8 and 10 dph and were able to ingest a 1st instar *Artemia* at 6 dph.

Finally the green water replacement trial showed that SanoLife ALG produced the highest survival ($28.6 \pm 9.2\%$), and there were no significant differences in growth among the algal products. The highest growth was seen in the clay treatments, although the lowest survival also came from the clay and tracer dye treatments (Table 2).

Larval Production: Survival was quantified for all 50 dph larvae and ranged from 2.5-5.8% from egg. Transfer of the larvae at 10 dph was based on the behavior of the larvae. From the time of eye pigmentation (2-3 dph) through 10 dph, the majority (70-80%) of the larvae were at the surface of the water allowing for easy transfer. Prior to transfer, swim bladder inflation was assessed and ranged from 50-100% among cohorts. There was no noticeable mortality seen from transfer of larvae at 10 dph; the majority of the larval mortality began to occur close to the completion of flexion (17-21 dph) and most of the mortality was associated with spinning larvae at the surface. The cause of the spinning was investigated but not determined. Losses after flexion, occurring between 35 and 50 dph, were the result of aggressive behavior that included cannibalism. No disease outbreaks were observed during the culture period, however the deformity level seen during 2009 and 2010 ranged from 25-40%.

Discussion

Egg production and quality: CYT broodstock in this study spawned between April and July when water temperatures were 16.5 - 22°C . It has been reported that CYT spawn off southern California between April and August when water temperatures are 17 - 21°C (Baxter, 1960; Sumida *et al.*, 1985). A similar temperature range for *S. lalandi* spawning has been reported by researchers in New Zealand (Moran *et al.*, 2007). Eggs were seen in the trap as early as 1600 and as late as 0100, and timing of spawning events got earlier as the season progressed. This has also been described in other CYT, with fish

Table 2. CYT larval rearing trials completed in 2009 and 2010.

Trial	Treatments	End of Trial (dph)	DW ($\mu\text{g} \pm \text{SD}$)	NL ($\text{mm} \pm \text{SD}$)	Survival ($\% \pm \text{SD}$)
Egg Stocking	50 eggs/L	10	205 $\pm$ 19	5.51 $\pm$ 0.38	6.8 $\pm$ 3.5
	100 eggs/L	10	215 $\pm$ 41	5.40 $\pm$ 0.35	15.3 $\pm$ 8.1
	200 eggs/L	10	195 $\pm$ 44	5.41 $\pm$ 0.36	7.0 $\pm$ 4.9
Rotifer Density	15 rotifers/ml	10	146 $\pm$ 20	5.06 $\pm$ 0.38	0.2 $\pm$ 0.2
	30 rotifers/ml	10	133 $\pm$ 12	5.05 $\pm$ 0.37	4.3 $\pm$ 6.2
	45 rotifers/ml	10	160 $\pm$ 20	5.18 $\pm$ 0.33	1.1 $\pm$ 1.3
Artemia Weaning	6 dph	14	685 $\pm$ 181	7.15 $\pm$ 0.65 ^A	6.0 $\pm$ 1.8
	8 dph	14	590 $\pm$ 131	6.60 $\pm$ 0.86 ^B	5.3 $\pm$ 3.4
	10 dph	14	530 $\pm$ 157	6.83 $\pm$ 0.73 ^B	5.3 $\pm$ 4.4
Green water Replacement	Live algae	10	340 $\pm$ 121 ^{AB}	5.81 $\pm$ 0.55 ^B	16.3 $\pm$ 0.9 ^B
	Algae paste	10	292 $\pm$ 64 ^B	5.75 $\pm$ 0.46 ^B	17.2 $\pm$ 9.9 ^{AB}
	Dry algae	10	270 $\pm$ 48 ^B	5.59 $\pm$ 0.44 ^{BC}	11.4 $\pm$ 6.1 ^B
	SanoLife (ALG)	10	350 $\pm$ 48 ^{AB}	5.79 $\pm$ 0.46 ^B	28.6 $\pm$ 9.2 ^A
	Clay	10	485 $\pm$ 87 ^A	6.23 $\pm$ 0.56 ^A	1.4 $\pm$ 0.6 ^C
	Tracer dye	10	284 $\pm$ 104 ^B	5.43 $\pm$ 0.45 ^C	1.1 $\pm$ 1.4 ^C

*Values within each experimental column sharing superscripts are not significantly different.

spawning just prior to dawn in the first half of the season and closer to dusk thereafter (Moran *et al.*, 2007). The reproductive behavior described here is also similar to what Moran *et al.* (2007) described for captive CYT in New Zealand, where a single male positioned himself beneath the female, his snout directly beneath the female's gonoduct.

Egg and oil diameters reported in this study were smaller than what has been reported for *S. lalandi* from wild populations in waters off southern California (egg diameter 1.44 mm; oil diameter 0.32 mm; Sumida *et al.*, 1985), and larger (1.1 mm) than what was reported by Kolkovski and Sakakura (2007). Measurements from Moran *et al.* (2007) showed egg diameters ranging from 1.33-1.50 mm and oil diameters ranging from 0.30-0.33 mm. Egg sizes can vary both within a species and between populations of the same species (Beacham and Murray, 1985; Brooks *et al.*, 1997). Egg diameter has been shown to decrease over the spawning season for serial spawners (Lavens *et al.*, 1999). This was not seen in 2009 or 2010; egg diameters remained constant throughout each season. However mean egg diameters in the 2010 season were larger than the mean egg diameters in 2009. The larger eggs did not yield better hatch and survival to first feeding

rates between 2009 and 2010. The mean hatch rates over the season were slightly higher than what has been reported for *S. dumerili* (65%; Jerez *et al.*, 2006). There was a seasonal variation with consistently poor hatching success occurring closer to the end of spawning season.

Larval Rearing Trials: There was no clear treatment effect when stocking up to 200 eggs/L. Stocking rates for other species can range from 10-500 eggs/L, depending on the species and the purpose of culture (Benetti *et al.*, 2008; Gimenez and Estevez, 2008; Moran, 2007). The current HSWRI stocking density of 100 eggs/L has consistently produced good survival through 10 dph. The results from this trial showed that a stocking density of 200 eggs/L does not influence growth and survival at 10 dph, but it is unknown whether the higher initial stocking density has a negative impact later in development due to the density of surviving larvae.

Live feed management is a critical area in the development of larval culture for any emerging species. The type, amount, and time needed to transition to and from each live feed are main components of high survival and overall production of quality juveniles. It was determined that CYT can be reared in the tank with levels of 15-45 rotifers/ml,

whereas previous HSWRI protocols called for a minimum of 30 rotifers/ml. Based on this research, we have been able to reduce our rotifer needs by over 30%, saving valuable resources. The weaning time onto 1st instar *Artemia* was also determined. Previous weaning schedules began 1st instar introduction at 10 dph, but it was found that not only could CYT larvae ingest *Artemia* at 6 dph, but there was increased growth associated with larvae fed at the earlier age. Interestingly, the weaning times are shorter than what has been previously reported for other *Seriola* spp. (Benetti, 1997; Garcia and Diaz, 1995; Moran, 2007)

The possibility of replacing algae as the turbidity agent may further increase growth and survival through the reduction of organic material added to the culture tank; limitation of microalgae paste has the potential to limit bacteria growth and minimize larval mortality due to disease or infection (Conceicao *et al.*, 2010). Significantly, it was shown that CYT do not need algae to maintain green water or turbid conditions. The use of SanoLife ALG, as well as the appropriate use of clay, has the potential to increase larval survival and quality at an early age.

Larval Production: HSWRI larval production prior to 2009 was highly variable, with overall survival ranging from 0.1-5.0 %. The culture protocol before 2009 called for larvae to remain in the rearing tanks for 30-35 dph (completion of flexion) before transport to a juvenile rearing tank. The culture protocols were shifted to early tank transfers at 10 dph because of larval behavior. Beginning at 2-3 dph the larvae began swarming at the surface of the tanks and staying within the first 20-25 cm of the water column. The larvae remained in large concentrations at the surface until 20-24 dph (8.58-10.71 mm NL), when the larger fish began to exhibit schooling behavior. This change in protocol helped to gain some consistency in survival between cohorts (2.5-5.8%); however more investigation is needed to reach higher overall survival.

Cannibalism and aggressive behavior was noticed from 35 -50 dph. Similar cannibalistic and aggressive behavior was described for both *S. quinqueradiata* and *S. dumerili*, with larger juveniles showing aggression toward smaller individuals (Sakakura

and Tsukamoto, 1999; Moran, 2007). Sakakura and Tsukamoto (1999) also showed that biotic factors, such as starvation and high densities, can accelerate the aggression of the dominant fish. The time period described above coincided with weaning off live food onto dry food, and cannibalism was alleviated once the fish were accepting the dry pellets with regularity. Deformity rates seen between 2009 and 2010 ranged from 25 to 40% depending on the cohort. The main deformity seen was cranial malformations. These cranial malformations can be caused by nutritional factors such as a fatty acid or vitamin C deficiency, a high DHA:EPA fatty acid ratio or by wall-nosing or darting into the tank wall (Cobcroft *et al.*, 2001; 2004). Further study is needed to determine the causes and solutions to these malformations.

Conclusion

Since establishing dedicated systems for conducting larval rearing experiments and simulated production runs, we have conducted numerous experiments focused on refining culture methods for larval CYT. These studies have greatly accelerated our understanding of the culture requirements for this high performance species, as well as highlighting important areas for future research. This research is providing the basis for ensuring successful CYT commercial culture in Southern California.

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Charting the Future of Aquaculture Feeds in the United States

Michael B. RUST*

Abstract : The development and expansion of farming of carnivorous fish species will be constrained by a limited supply of fishmeal and fish oil for feeds. There is no dietary requirement for specific amounts of fishmeal or fish oil for fish, so feeds that lessen the reliance on these limited feedstuffs – such as alternative protein and oil resources – can, and must, be developed. For this reason, the U.S. Department of Commerce (NOAA) and the U.S. Department of Agriculture (USDA) jointly sponsored an aquaculture feeds initiative to address those issues. The initiative used expert and public consultations to identify and discuss the future of fish feeds and the benefits to the U.S. by the development of such alternative feeds. The resulting report from these meetings was released in draft form for comment at the end of 2010 and was finalized in 2011. This paper covers the processes taken to arrive at the recommendations. The report is available at <http://aquaculture.noaa.gov>

The report calls for feeds to be evaluated not only for nutritional and economic performance as is done today, but also for environmental and human health performance by taking into account the environmental footprint of feed production and use, and the resulting quality of the product for human consumption. This “triple bottom line approach” – economics, environment and human health – is supported by 20 specific recommendations. Implantation of these recommendations has already started in some cases, but will take time to be fully developed.

Keywords : aquaculture feeds initiative, economics, environment, human health

Introduction

To meet the growing consumer demand for seafood in the United States, increasing supplies of finfish and shellfish will be needed. Most experts agree that development of aquaculture will be the only way to meet this increase in demand. The challenge is how to ensure that aquaculture production increases are sustainable.

For example, the development and expansion of farming of carnivorous fish species such as salmon may soon be constrained by a limited supply of fishmeal and fish oil for feeds. Today, salmon is the third-highest consumed fish in the United States and roughly seventy percent of the world's supply of

salmon is now farmed.

Fishmeal and fish oil have traditionally been used to make up a large part of the diet of farm-raised salmon. The composition of these feed ingredients is almost perfectly matched to the dietary requirements of carnivorous fishes. There is no dietary requirement for fishmeal or fish oil for salmon or any other fish. The dietary requirements are for the nutrients they contain (amino acids, fatty acids, vitamins and minerals) not the ingredients *per se*, so feeds that lessen the reliance on these limited ingredients – such as alternative protein and oil sources – can be developed.

For this reason, the U.S. Department of Commerce (NOAA) and the U.S. Department of Agriculture

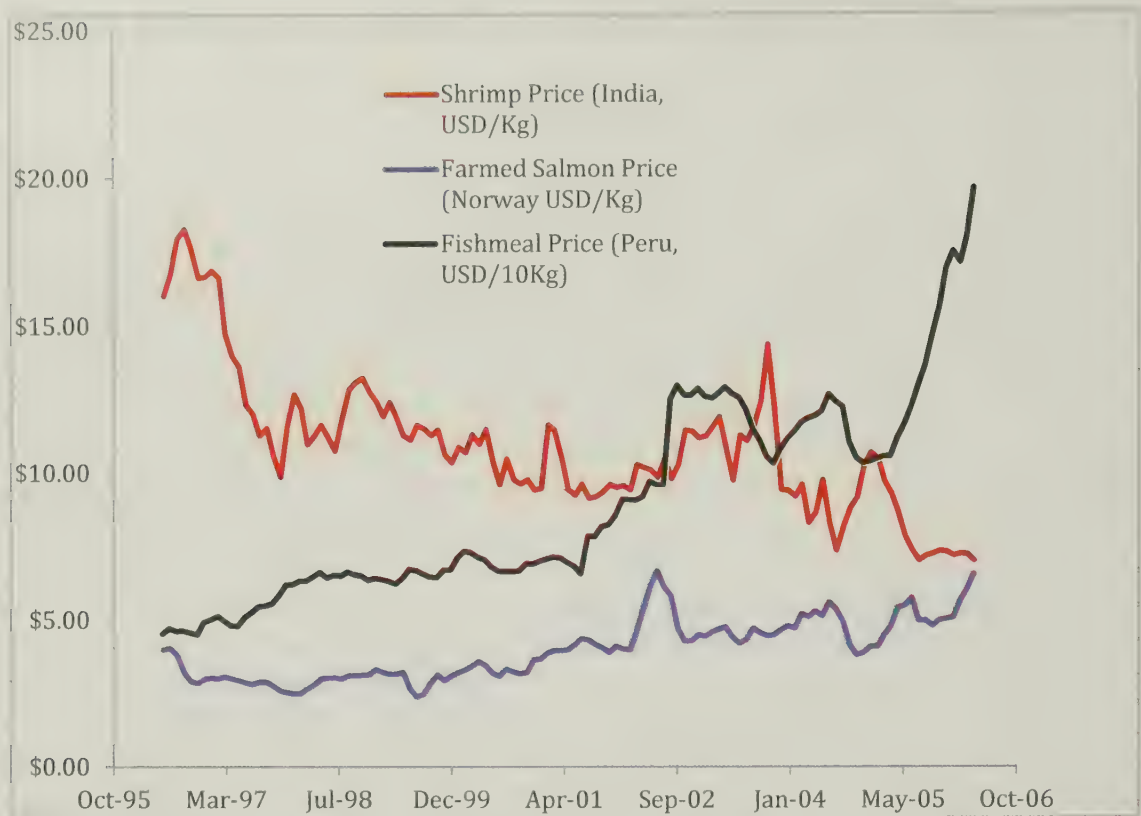


Fig. 1. World price for fishmeal, farmed shrimp and farmed salmon from 2000 to 2010.

(USDA) sponsored expert and public consultations on the future of fish feeds and the benefits to the U.S. economy by the development of such alternative feeds. Those agencies were greatly aided in this effort by help and advice from scientists and others within the Department of the Interior (DOI) and the Food and Drug Administration (FDA).

The Consultation Process

The consultation consisted of eight parts:

1. An invitation to the general public to comment on the issue, and respond to four questions designed to elicit input on a broad array of topics and approaches was published in the Federal Register. The public comment also helped indicate the level of understanding and knowledge that the public has regarding fish feeds.
2. A consultation with experts who are active researchers in the area of fish feeds,

feedstuffs, nutrition, and related topics (Research Experts Panel).

3. A consultation with experts who are active stakeholders in the area of fish feeds, feedstuffs, nutrition, and related topics (Stakeholder Experts Panel).
4. Reporting case studies where the shift from reduction fishery fishmeal and fish oil to alternatives is already happening or are areas that might hold promise for the future.
5. Future-casts focused on fish feeds by the attendees at the two experts meetings.
6. Information and recommendations from the above were summarized in a Draft Report addressing the questions raised by the public comment process, summarizing the results of the two experts panels, and reporting on the case studies.
7. A public review of the Draft Report to provide final input from the public and interested groups before it is finalized.

8. Publication of the final Report and outreach to interested parties.

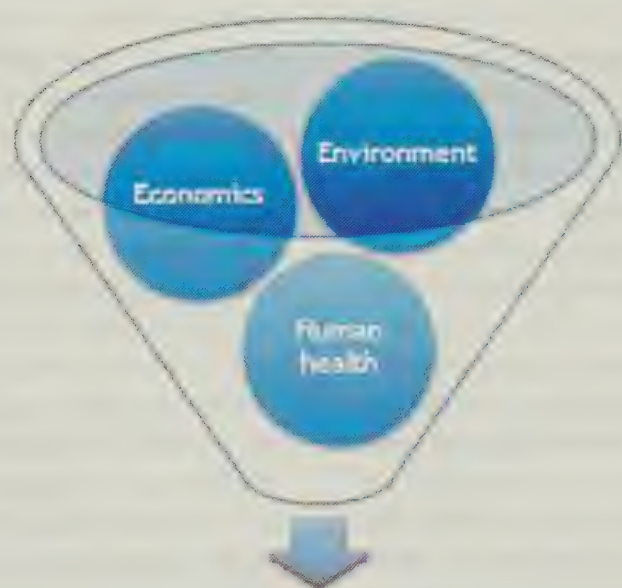
A Steering Committee made up of scientists with expertise in fish nutrition, federal policymakers, and communications experts was assembled to move the process forward.

The purpose of the Steering Committee was to fine-tune the objectives and questions asked, suggest and contact the appropriate scientists, develop dates and locations for panel meetings, choose facilitators for panel meetings, serve as reviewers of the Report, and develop presentations to be given at public meetings. The editorial sub-committee assembled, wrote portions of, and edited portions of the Report. Numerous authors contributed case studies in their areas of expertise and produced future-casts.

In conducting this initiative, the steering committee was guided by several principals when considering an ingredient, process or approach to reduce the use of conventional fishmeal and oil in

aquaculture:

- The committee was more interested in what to do, rather than what not to do. Thus all ideas were welcomed equally. Conversely, objections to various feedstuffs were recorded but this was not viewed as justification for their exclusion from consideration or exclusion from the final white paper.
- The committee attempted to adopt a triple bottom line approach when evaluating alternatives. This meant trying to account for:
 - The economic performance of an ingredient, process or approach,
 - The environmental performance of procuring an ingredient, employing a process, or following an approach and,
 - The human health performance of the product resulting from the substitution of an ingredient, process or approach.



Feeds for Healthy Sustainable Aquaculture

Fig. 2. The “triple bottom line” approach to future aquafeed development.

Public Comment

The Federal Register notice containing questions to solicit input was published November 16, 2007. The public comment period ended February 29th 2008. The questions from the FR notice are:

- 1) Where should the federal government focus its research efforts in the area of alternative feeds for aquaculture? Are there specific areas that the federal government should not address?
- 2) What are potential alternative sources of protein and oil for aquaculture feeds? For example, are there specific opportunities for greater use of seafood processing waste and other agricultural by-products in aquaculture feeds? Are there specific obstacles to using these alternatives as alternative dietary ingredients in aquaculture feed?
- 3) What type of treatments or processes show promise for improvement of existing aquaculture feedstuffs and for developing new feedstuffs? How soon could these technologies be commercialized?
- 4) Fishmeal and fish oil contribute important human nutritional components to aquaculture feeds, such as omega-3 fatty acids. As the aquaculture feeds industry seeks to replace fishmeal and fish oil with alternatives, how can the nutritional benefits of farmed seafood be maintained or enhanced? For example, what technologies exist for producing omega 3 fatty acids?

The public submitted over 40 separate comments in the following areas: 1) Products for sale, 2) Questions, 3) Statements, 4) Priorities, and 5) Ideas. These comments ranged from very well developed lengthy letters, to one-line statements. The small number of comments makes further analysis difficult.

Experts Panels

Two groups of experts were assembled to develop a path to the development of commercial diets that have no net usage of fishmeal or oil derived from commercial pelagic fisheries. The

first panel (Research Panel) was made up of 21 scientists actively working and published in feeds and feedstuffs research, fish and human nutrition, bio-energy, processing, agriculture, and related areas. These scientists provided an unbiased basis for further development. The panel was primarily made up of university and government scientists from around the world. Scientists came from Australia, Canada, Japan, Norway and the United States. This panel met at NOAA's Manchester Lab in Washington State in February 2008.

The second panel (Stakeholders Panel) was made up of individuals who are experts in the practical areas of feeds, human nutrition, and specific feedstuffs. The membership included environmental groups, consumer groups, public hatchery system managers, the commercial aquaculture industry and others with expertise related to the topic. They addressed the same charge as the research panel. This panel met at NOAA headquarters in Silver Spring, MD, in April, 2008.

The expert panel workshops were used to capture expert opinions, develop consensus on key issues where possible, and vet options. Observers included government officials who are responsible for setting research funding priorities, regulators and policy makers at the agencies with interest in feeds for aquatic organisms.

Both panels had the same four assignments:

- 1) Answer the questions resulting from the Federal Register announcement.
- 2) Identify the constraints and possible solutions to providing aquafeeds in the future as fishmeal and fish oil resources become scarce.
- 3) Identify key research and technology transfer needs to overcome barriers for reducing reliance on fishmeal and fish oil resources.
- 4) Predict (forecast?) the future of aquaculture feeds based on 1), 2), and 3).

Case Studies

The steering committee solicited short write-ups from individuals who are already actively working to replace fishmeal and oil, on case studies of concrete examples of research leading to replacements, actual replacements, or areas that could hold great promise

for replacement in the future.

In total, seven case studies were developed. These case studies covered the use of terrestrial plant diets, soy, seafood processing waste, macroalgae, and the importance of understanding nutrient requirements. Case studies also considered freshwater and marine fish, as well as marine shrimp.

Future-casts

Future-casts are a bit of “science fiction.” The convening of both the Researchers Panel and the Stakeholders Panel provided the opportunity to ask each participant to provide a vision of the future of aquaculture feeds as they saw it. At the end of each workshop, attendees were asked to spend some time thinking about and recording what they see as the state of feeds for aquaculture in the future. Specifically, participants were asked to predict the challenges and changes that aquaculture will face, and the developments that will affect both producers and consumers over the next five and 25 years. Participants in each panel varied widely in background and expertise and represented multiple viewpoints providing a variety of visions of the future. Since this was an optional homework assignment, the number of future-casts is smaller than the number of participants in the panel workshops. The future-casts provide an opportunity for those who have an interest in the future of aquafeeds to distill current trends, issues, and science into a plausible vision for the future of aquaculture feeds.

In total, 12 future-casts were submitted. One condition was common to all scenarios and future-casts; human population growth continues and the demand for more fish and seafood increases. The need for aquaculture research to develop sustainable sources of nutrients and develop farmed animal populations that can thrive on a variety of nutrient sources is vital.

Draft Recommendations

The draft Report was released for final public comment late in 2010. Final public comment was due by spring 2011. Barring any major issues discovered

during the final public comment period, the report contains the following 20 recommendations.

Fishmeal and fish oil are not nutritionally required for farmed fish to grow: About 40 nutrients – essential amino acids, vitamins, minerals, and fatty acids – are required, but they can be obtained from sources other than fishmeal and fish oil. Fishmeal and fish oil have been the preferred ingredients in fish feeds because they contain the nutrients in nearly perfect balance, are easily digestible by the fish, result in good growth and survival, and provide human health benefits. Combining other ingredients to get the same balance is possible, but will require fully understood fish requirements and alternative performance.

Farming of fish is a very efficient way to produce animal protein and other human nutritional needs: Farmed fish use their feed very efficiently. For example, farmed Atlantic salmon can convert approximately one kilogram of feed (dry) into one kilogram of flesh (wet). In contrast, the feed conversion of poultry is about 3:1, and pork is about 6:1. Fish need fewer calories because they are cold-blooded and do not need to support their weight.

Feed manufacturers making diets for carnivorous fish and shrimp have already reduced their reliance on fishmeal and fish oil: Application of previous research led to cost-effective substitution using alternatives, which helped mitigate feed costs in the face of increasing fishmeal prices (see Figure 1). In the past 15 years the ratio of fish in to fish out has dropped from 3-4:1 to approximately 1.5:1 for major aquaculture species due to increased use of protein and oils in diets from non-marine sources. Fishmeal and fish oil are likely to be increasingly reserved for use in specialty diets (broodstock and larval diets) and finishing diets to maintain the human health benefits from farmed seafood.

Economics is currently the major driver of using alternate feed ingredients in feed mills: Feed producers make substitutions for fishmeal and fish oil according to how their price compares with allowable alternatives (i.e., alternatives for which sufficient nutritional and production knowledge and experience exists to allow their use). Panels identified some crucial factors limiting changes to

feed formulations, including insufficient information on nutrient requirements of farmed species – especially newly domesticated species – and on available nutrient content and nutritional value of alternative ingredients for fish and shrimp. This area requires investments in research to help feed producers understand the costs and benefits of including alternative ingredients in aquaculture feeds.

The net environmental effects of the production and use of alternate feeds should be considered: Consideration should be given to the environmental impacts of making dietary changes to feeds for farmed aquatic organisms.

The human health implications of using alternative feeds needs to be better understood and considered: Long chain omega-3 fatty acids and other nutritional compounds found in fishmeal and fish oil provide important human health benefits. Seafood reared on alternative feeds must continue to provide these health benefits to consumers. Human health considerations should be addressed along with economic and environmental considerations when alternatives are considered. To accomplish this, fish nutritionists should work with human nutritionists and food scientists on promising alternative ingredients to determine impacts of those alternatives on final product quality.

Fishmeal and fish oil are minor contributors to the world protein and edible oil supply: In 2007, fishmeal accounted for approximately 2.3% of total protein meals and fish oil for about 2.0% of total edible oils. The largest supply of protein on Earth is from soybeans. A 4% increase in soy protein meals would nearly equal the total world fishmeal supply. An increase in the amount of soy protein equal to world fishmeal annual production has been achieved about every five years without any additional cropland, based on historical increases in yield per unit area due to intensification, new cultivars, and altered farming practices.

Recovery and utilization of fisheries processing waste should be encouraged and increased: Processing waste has been shown to produce products of similar biological value to fishmeals and fish oils obtained from industrial fisheries. The total worldwide amount of fish processing waste

from wild capture and aquaculture may equal the amount of forage fish used for fishmeal and fish oil from industrial fisheries. But fish processing waste is often not economical to obtain because of logistical and technical constraints. Research and financing is needed to help capture the waste products from wild capture fisheries that often are located in remote or inaccessible regions with poor infrastructure. Likewise, research to capture and reuse the waste products from aquaculture should be undertaken. The use of processing waste from aquacultured organisms to produce fishmeal and fish oil eventually could make aquaculture a net producer of fishmeal and oil.

Plants produce the vast majority of protein and edible oils in the world, accounting for 94% of total protein production and 86% of total edible oil production: Plants also make up a substantial proportion of diets for carnivorous fish (e.g., 50-60% of a typical salmon diet). It is likely that plants will deliver the bulk of the amino acids and fats in farmed fish feeds in the future due to abundance, the potential for increased production, and low cost. Research to increase the use of sustainable plant products in feeds for aquatic organisms will help increase the importance of agriculture to aquaculture and vice versa. This area of research would be as important to farmers as to aquaculturists and may represent a significant opportunity for American farmers.

Algae-based biofuel may present opportunities for feed ingredient production because protein is a byproduct of oil recovery from algae, and marine algae produce the long chain omega-3 fatty acids and certain amino acids important to fish and human health: It is too early to understand the ramifications of increased algae biomass production for fish diets, and this area will require communication between algae biofuel scientists and fish nutritionists. Support of research in this area is justified for producing the long chain omega-3 fatty acids alone, as they are a potentially higher value product than biofuel.

There will likely be increased demand for and production of ethanol and bioplastics, and byproducts from those industries could make good ingredients for fish diets: Fish feeds are mostly made up of protein and oils. Ethanol and some bio-plastic

are made from the carbohydrate fraction of plants, leaving behind the protein and oils. Future biofuel production may be quite different from today's focus on ethanol made from corn carbohydrates, which uses a process that degrades the quality of protein waste products. If grain remains a feedstock for ethanol production, new approaches to recover high-quality protein and oil from the ethanol production process will be needed to make it suitable for widespread use in fish feeds. Biodiesel is made from the oil fraction, leaving behind concentrated protein that is already suitable for fish. Fish nutrition researchers should work and coordinate with biofuel scientists to ensure that byproducts are safe and usable for fish. Research that supports processes resulting in high-quality protein and oil byproducts from fuel production should be encouraged.

As replacements, many alternatives are higher in cost per unit fish gain (biological value) than fishmeal and fish oil: However, the recent trend (since 2006) has been for fishmeal and fish oil prices to increase faster than the prices of alternative protein and oil sources. Research that can help lower costs or improve the biological value, without raising costs, will increase the rate of fishmeal and fish oil replacement.

Fish have dietary needs and preferences for specific compounds not found in plants, so there is a need for specialized products that supply those compounds and/or add flavor to the diet: These ingredients will likely be higher in cost than the bulk protein and oil products and will need to contain flavors, nutrients, or other properties not found in bulk proteins and oils but which are needed for fast growth, health, or increased consumption. Examples are algae, invertebrates, animal by-products, and seafood trimming meals and oils. Additional ingredients such as immune system enhancers are also beneficial to enabling the use of higher levels of alternatives. Research is needed to develop materials that will enable greater use of cheaper, more abundant protein meals and oils.

Alternative sources of protein and oil are common commodities used in livestock and companion animal feeds and come from novel byproducts from other industries, underutilized resources, or completely novel products: Existing commodities that

have the potential for greater use in feeds include protein concentrates from grains or oilseeds and byproducts from animal proteins. Novel byproducts from other industries include proteins recovered from biofuel production or single-cell proteins produced from inexpensive carbon sources. Other sources include fish processing wastes, trimmings, and/or bycatch from fishing.

New products include meals produced from worms, insects, and marine invertebrates, and meals and oils from algae. What these products have in common is that they are underused and/or underdeveloped protein and oil sources that require variable degrees of investment in research and development to become more widely employed as feed ingredients. Some possess components that are detrimental to fish (e.g., anti-nutrients), or they contain insufficient levels of essential or semi-essential nutrients and need to be processed, blended with complementary products, or supplemented. More information is also needed to evaluate the environmental impacts associated with using various feed ingredients. Information on contaminant content of alternate products is also needed to place risks and benefits to fish wellness and human health into a rational context. Coupled with this is the opportunity to maintain or improve the safety and healthfulness of farmed fish products for the consumer by using alternate ingredients. All these topics will require investments in research and development.

Plants and other alternatives contain some compounds (anti-nutrients) that are detrimental to fish: Although there are processes to remove or inactivate many of these compounds, further research and development is necessary to improve those processes. Fish may also be selectively bred to be more tolerant of the anti-nutrients in present in some alternatives.

Harvest of lower trophic level species, such as krill, for meal and oil production may be possible, but the environmental benefits afforded to the marine ecosystem from those species should be considered along with the economic and nutritional aspects of their use: While this may provide an option in the near term, the harvest of any wild population, including krill, would require careful management

and would be limited to what nature can supply.

The use of bycatch for production of fishmeal and fish oil could provide a substantial amount of those products without increasing the current impact from the wild capture fisheries: Although traditional processes exist to convert bycatch into fishmeal and fish oil, concerns over creating a market for non-target species and the logistical issues associated with dealing with retained bycatch at sea have been expressed.

Demand for long chain omega-3 fatty acids for both direct human consumption and feed ingredients is likely to increase beyond the amounts available from marine resources: Alternative sources such as algae, microorganisms, and/or oilseeds are needed and should be developed. More efficient use of long chain omega-3 fatty acids can be made in aquaculture through improvements in feeding practices and formulation. Research leading to new cost-effective sources of long chain omega-3 fatty acids will benefit human health as well. Research to improve production and the efficiency of use should also be supported.

Farmed fish species are being increasingly domesticated and performance is improving through conventional genetic selection for performance on plant-based and other non-fish based aquafeeds: As aquatic species are domesticated, selection can be directed toward better use of non-fishmeal and non-fish oil ingredients.

Scientific information on the nutritional requirements of farmed fish species, feed ingredients, and the interaction between the fish and the diet will need to expand greatly to make substantial improvements in feed formulation by commercial aquaculture feed producers: Updating the National Research Council (NRC) requirements for fish on a regular basis and support for research that helps define the basic nutritional requirements for farmed aquatic species should be supported.

Annotated Bibliography of Key Works

Mozaffarian D. and Rimm E., 2006: Fish intake, contaminants, and human health: Evaluating the risks and benefits. *JAMA*, **296** (15), 1885-1899.
The authors for the first time present a

comprehensive human health model based on concentrations of mercury, dioxins, polychlorinated biphenyls, and long chain omega-3 fatty acids for fish and project the impact of increased seafood consumption in the U.S. on the population's health. This model accounts for the increased risks associated with consumption of contaminated seafood along with the benefits from increased consumption of long chain omega-3 fatty acids. Overall the authors predict that increasing the per capita annual consumption of seafood in the U.S. from the current 7.25 to 11.8 kg per person (1-2 servings per week of species high in omega-3 fatty acids) would result in a decrease in coronary death by 36% and an overall decrease in total mortality of 17%. Further the authors provide the amounts to consume of various species and the cost to provide the benefits associated with seafood consumption. Implications for target nutrient and contaminate levels in aquacultured fish can be derived from the information presented in this paper.

Rust M. B., Barrows F. T., Hardy R. W., Lazur A., Naughton K., and Silverstein J., 2010: Draft NOAA/USDA Alternative Feeds Initiative: The future of Aquafeeds. *Agency Technical Report*, available at <http://aquaculture.noaa.gov>.

This report is the subject of this presentation. More details and recommendations are found on the US approach to future development of diets for aquaculture.

Tacon A. G. J., and Metian M., 2008: Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. *Aquaculture*, **285**, 146-148.

This paper provides a peer-reviewed source of information on aquaculture use of fish meal and fish oil up to the mid 2000's with projections into the future. Environmental implications to the overuse of stocks of industrial fish are discussed.

Progress of DNA Marker-Assisted Breeding in Maricultured Finfish

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Abstract : The marine products industry has developed based on direct catch from natural resources. However, because of the depletion and gradual restriction of aquatic resources, only recently has breeding been considered as an important research area. With the depletion of aquatic resources, the expectation regarding aquaculture research is increasing. Also genetic improvement of traits for improved culture is leading to superior varieties because we can improve the phenotype to suit aquaculture conditions by domestication of artificially produced fish with each generation.

The essential conditions for DNA marker-assisted selection (MAS) is development of useful resource families to evaluate phenotypes and information about genetic linkages and a large number of polymorphic genetic markers. At the beginning of the study, we mainly aimed to use improved technologies on salmonid fish (Ozaki *et al.*, 2001). But now the research and development are being applied to other kinds of maricultured fish. Some of the cases have already reached a practical stage and have been used in genetic improvement production. Two cases of MAS programs have succeeded in developing Japanese flounder resistant to lymphocystis disease (Fuji *et al.*, 2007) and Atlantic salmon resistant to infectious pancreatic necrosis (Moen *et al.*, 2009). These cases indicate the validity of the methodology.

MAS breeding is applicable to other target species. However, there still remain problems and potential to further improve the methodology. One is markers-assisted introgression (MAI) or quantitative trait loci (QTL) pyramiding. MAI is the next phase of MAS. This method uses DNA markers of the responsible region for specific traits, and introgression hybridization to obtain several economic traits on one strain. We are carrying out research and development about MAI using Japanese flounder (*Paralichthys olivaceus*) as a target species. The other is effective utilization of natural genetic resources. At this point, aquatic resources have advantages because wild species have not been selected and still maintain high genetic diversity. Individuals have high potential for genetic breeding regarding phenotypic characters. We are researching practical applications about selection of economic important traits from natural genetic resources using Japanese amberjack (*Seriola quinqueradiata*) as a target species.

Keywords : marker-assisted selection (MAS), markers-assisted introgression (MAI), DNA marker, economic traits

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Introduction

The marine products industry has developed based on direct catch from natural resources. However, because of the depletion and gradual restriction of aquatic resources, only recently genetic breeding has been considered as an important research area. With the depletion of aquatic resources, the expectation regarding aquaculture research is increasing. Also genetic improvement of traits for improved culture is leading to superior varieties, because we can improve the phenotype to suit aquaculture conditions by domestication of artificially produced fish with each generation.

The essential conditions for DNA marker-assisted selection (MAS) is development of useful resource families to evaluate phenotypes and information about genetic linkages and a large number of polymorphic genetic markers. At the beginning of our research, we mainly used improved technologies on salmonid fish. But now the research and development are being applied to other kinds of maricultured fishes. Some of the cases have already reached a practical stage and have been used in genetic improvement production. Two cases of MAS programs have succeeded in developing Japanese flounder resistant to lymphocystis disease and Atlantic salmon resistant to infectious pancreatic necrosis (IPN). These cases indicate the validity of the methodology.

MAS breeding is applicable to other target species. However, there remains the potential to further improve the methodology. One is markers-assisted introgression (MAI) or quantitative trait loci (QTL) pyramiding. Markers-assisted introgression is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits and introgression hybridization to obtain several economic traits in one strain. We are carrying out research and development on MAI using Japanese flounder (*Paralichthys olivaceus*) as a target species.

The other improvement is effective utilization of natural genetic resources. At this point, aquatic resources have more advantages because wild species are not selected and still maintain high genetic diversity. Individuals have high potential

for genetic breeding with respect to phenotypic characters. Aquaculture species have those genetic resources. We are researching practical applications for selection of economic important traits from natural genetic resources using Japanese amberjack (*Seriola quinqueradiata*) as a target species.

First Case of Identified Loci Associated with Disease Resistance and Pilot Examination of Marker-assisted Selection

IPN is a well-known acute viral disease of salmonid species. We have identified QTLs associated with resistance to this disease in rainbow trout (Ozaki *et al.*, 2001). We searched for linkage among 51 microsatellite markers used to construct a framework linkage map in backcrossed families of rainbow trout (*Oncorhynchus mykiss*) produced by crossing IPN-resistant (YN-RT201) and IPN-susceptible (YK-RT101) strains. Two putative QTLs affecting disease resistance were detected on chromosomes A (IPN R/S-1) and C (IPN R/S-2; Figure 1), suggesting that this is a polygenic trait in rainbow trout. These markers have great potential for use in MAS for IPN resistance and provide the basis for cloning of IPN resistance genes. Clarification of the genetic bases of complex traits has broad implications for fundamental research, but will also be of practical benefit to fish breeding.

In our analysis of IPN disease resistance/susceptibility, we showed the possibility of MAS; therefore, we performed MAS in a candidate locus (IPN R/S-1) that was related to IPN disease resistance in the analysis family. Progeny obtained from the crossbreeding were tested with artificially induced infection. As a result, the combination of crossbreeding that had the positive allele of YN-RT201 showed significantly higher resistance compared to the negative allele (Figure 2).

Marker-assisted Selection and MAS-lymphocystis Disease Resistant Flounders Are Now Marketed in Japan

An allele of a microsatellite, Poli9-8TUF (Figure 3), has a dominant effect at a single major locus and is responsible for resistance to lymphocystis disease

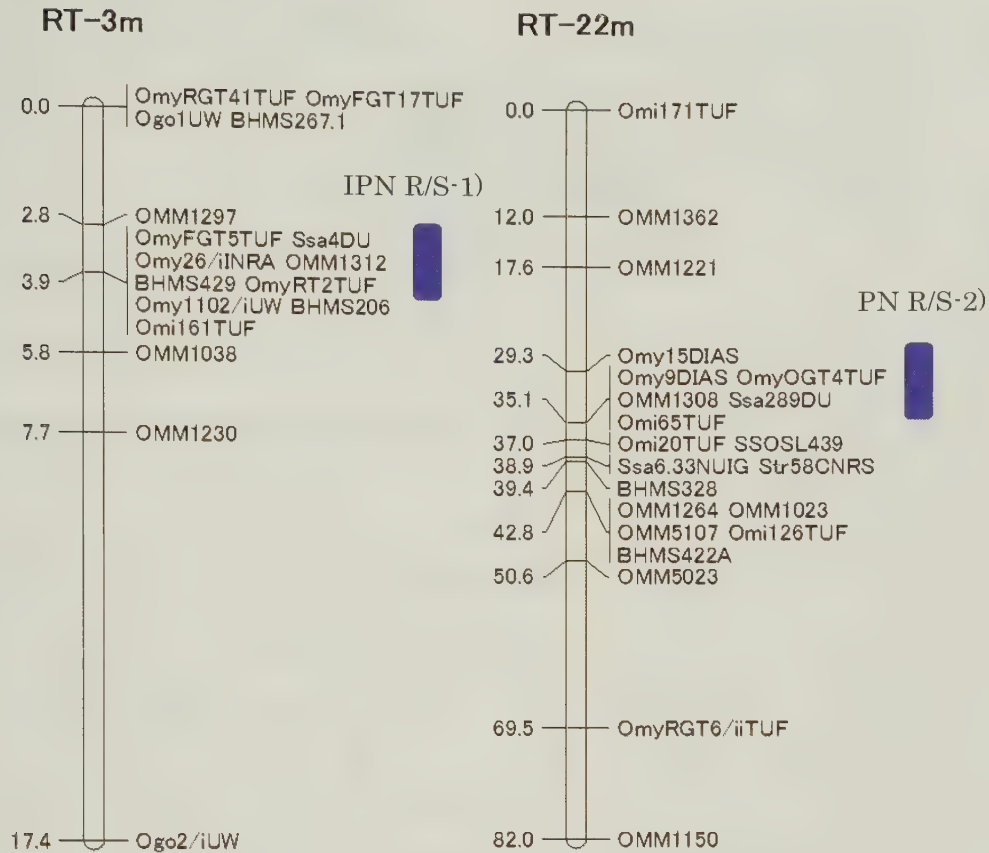


Fig. 1. Genetic linkage maps of significant loci in linkage group RT-3 and RT-22 in rainbow trout. Map distances between markers are shown in centimorgans (cM). A LOD of 3.0 was used to create linkage groups of each family. Map figures were drawn using MapChart ver.2.0.



Fig. 2. Five days after initiation of the infection experiment with infectious pancreatic necrosis virus (IPNV) in rainbow trout. The positive allele on IPN R/S-1 clearly showed IPN disease resistance. Fish on the left have the positive allele have; those on the right have the negative allele.

(LD-R) in Japanese flounder. A new population of Japanese flounder was produced by marker assisted breeding using this allele. A female that originated from the KP-B inbred line with LD-R that was homozygous for the favorable allele (B-favorable) and a male from a commercial stock bred for higher growth rate and good body shape were selected as parents. A female was selected as the LD-R-bearing parent because the recombination rate of females is lower in the region where the LD-R locus is located. As expected, the B-favorable allele was transmitted as a heterozygote to the progeny (LD-R+ population). The LD-R+ population, when tested at two commercial fish farms that had LD outbreaks, showed no incidence of LD at either farm, while a control population without B-favorable alleles (LD-R-) had incidences of 4.5% and 6.3% at the two farms. These results show that marker-assisted breeding using molecular markers linked to an economically important trait is an efficient strategy for breeding (Fuji *et al.*, 2007). MAS-lymphocystis disease resistant flounder are now selling in marketplace and have a market penetration rate 35% in Japan (Figure 4).

Linkage Analysis of Resistance to *Streptococcus iniae* Infections in Japanese Flounder (*Paralichthys olivaceus*)

Streptococcal disease caused by *Streptococcus iniae* is a serious bacterial disease in Japanese flounder. Developing streptococcosis-resistant Japanese flounder will reduce the number of outbreaks of the disease as well as reduce the need for antibiotics and vaccines. Genetic linkage analysis is an effective method for identifying QTL associated with resistance to a disease. In this study, 159 microsatellite markers selected from genetic linkage maps of Japanese flounder and F1 progeny from crosses between disease-resistant and disease-susceptible parents were used for detection of QTL associated with resistance to the disease. Some loci associated with disease resistance were found in the JF-7, JF-10, JF-11 and JF-17 linkage groups (Ozaki *et al.*, 2010). These QTL regions are candidates for disease resistance against streptococcal infection.

MAS breeding has the potential to further improve the methodology through MAI or QTL pyramiding. MAI is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits and introgression hybridization to obtain several economic traits in one strain. We are carrying out research on and development of MAI using Japanese flounder as a target species, to make the strain disease resistant and to incorporate other positive traits (Figure 5).

MAS Programs Have Succeeded in Atlantic Salmon Resistant to IPN in Norway

IPN is an economically devastating disease in Atlantic salmon (*Salmo salar*) farming worldwide. The disease causes large mortalities at both the fry and post-smolt stages. Family selection for increased IPN resistance is performed through the use of controlled challenge tests, where survival rates of sib groups are recorded. However, since challenge-tested animals cannot be used as broodstock, within family selection is not performed and only half of the genetic variation for IPN resistance is being exploited. DNA markers linked to QTL affecting IPN resistance would therefore be powerful selection tools. The aim of this study was to identify and fine-map QTL for IPN-resistance in Atlantic salmon for use in MAS to increase the rate of genetic improvement for the trait (Moen *et al.*, 2009).

The QTL confirmed in this study represents a case of a major gene explaining the bulk of genetic variation for a presumed complex trait. QTL genotypes were deduced within most parents of the 2005 generation of a major breeding company, providing a solid framework for linkage-based MAS within the whole population in subsequent generations. Since haplotype trait associations valid at the population level were found, there is also a potential for MAS based on linkage disequilibrium. However, in order to use MAS across many generations without reassessment of linkage phases between markers and the underlying polymorphism, the QTL needs to be positioned with even greater accuracy. This will require higher marker densities than are currently available.

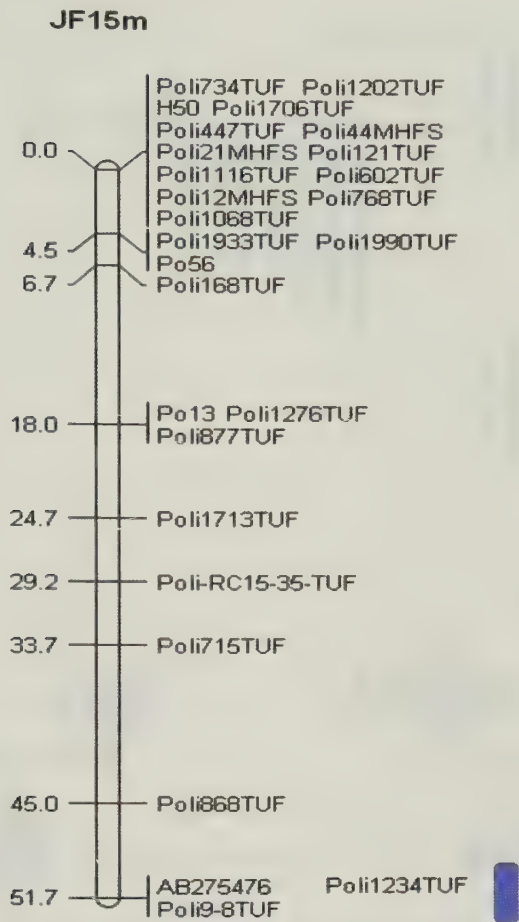


Fig. 3. Genetic linkage maps of significant loci in linkage group JF-15 in Japanese flounder. Map distances between markers are shown in centimorgans (cM). A LOD of 3.0 was used to create linkage groups of each family. Map figures were drawn using MapChart ver.2.0.



Fig. 4. MAS-lymphocystis disease resistant flounder have a market penetration rate of 35% in Japan.

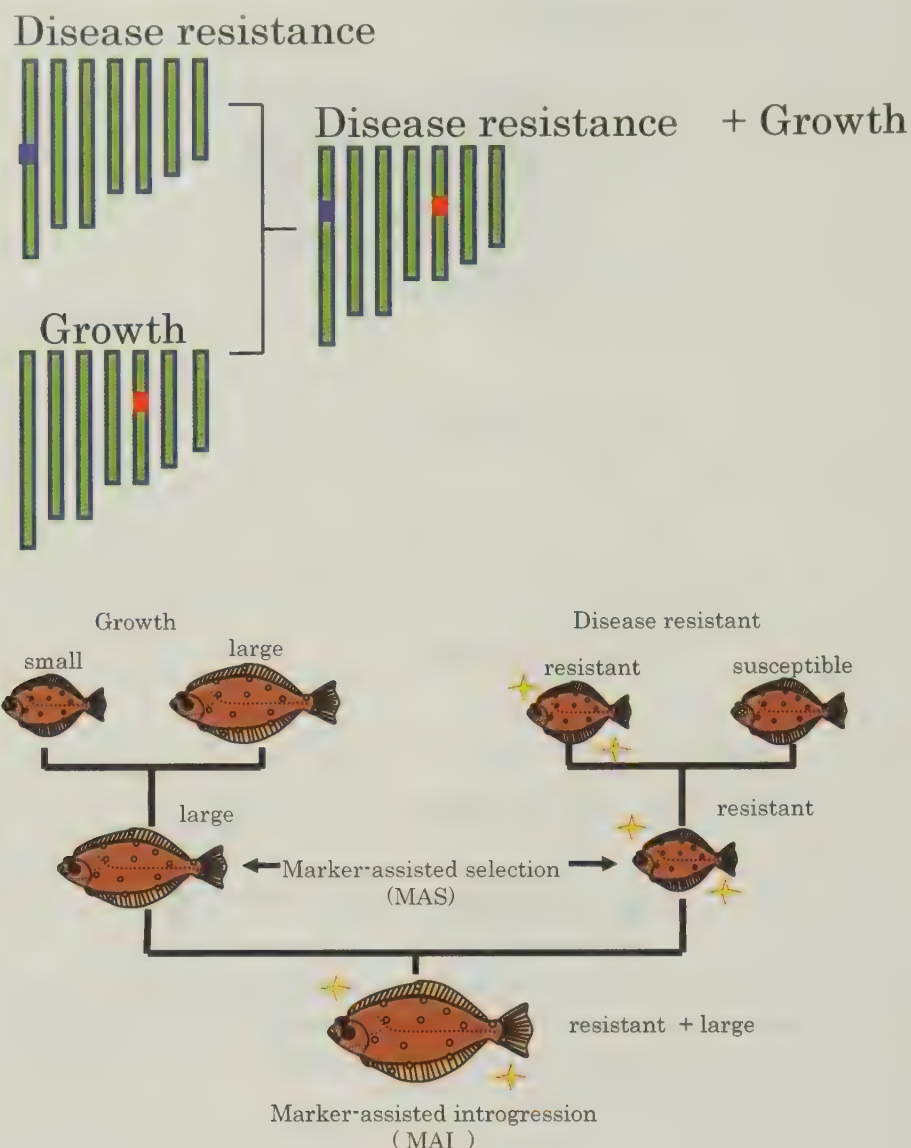


Fig. 5. Markers-assisted introgression is the next phase of marker-assisted selection. This method uses DNA markers of the responsible region for specific traits, and introgression hybridization to obtain several economic traits on one strain.

Developing the Infrastructure of New Molecular Tools and Information Using Single Nucleotide Polymorphism and Parasitic Disease Resistance QTL Analysis in Yellowtail

Yellowtail (*Seriola quinqueradiata*) is one of the most important commercial aquaculture species in Japan. However, most of the production facilities use captured juveniles. In particular, establishment of hatchery-produced juveniles are desired for

sustainable aquaculture production systems. That can make discovery of economically important traits from genetic resources possible. We are trying to develop the infrastructure of new molecular tools and information using single nucleotide polymorphism (SNP).

The sex-linked locus in yellowtail has been identified using microsatellite markers. We characterized the sex determining system by genetic linkage analysis conducted on fish of both sexes from

a single family. The associations between phenotypic sex and genotypic data of microsatellite markers selected from a yellowtail genetic linkage map were tested. The putative sex-determining locus is located between locus Sequ21 and locus Sequ17 in LG12, and the sex-linked alleles were inherited from the female parent(Fig. 6). This result suggests that yellowtail has a ZZ – ZW sex determining system, and that it would be possible to use these sex-linked markers to discriminate the sexes (Fuji *et al.*, 2010).

The parasitic disease benedeniasis is a serious problem in *Seriola* species caused by *Benedenia seriolae*. The parasite can cause growth reduction and external injuries in yellowtail, increasing the risk of secondary viral or bacterial infections. We produced F₁ hybrids in yellowtail and also constructed genetic linkage maps using microsatellite markers. In addition, we are trying to identify QTLs associated with resistance to *B. seriolae* by those molecular markers in F₁ and F₂ hybrids.

Genetic analysis is performed to detect loci associated with phenotype as a step toward new genetic breeding programs. Also, marker-assisted and marker-introgression breeding programs have attracted a great deal of attention for their use in improving multiple economically important characters. Haplotype genetic selection has just started as is limited to a few fish species for which control of the life cycle has been completed. It will make it possible to do tailor made genetic breeding using genotype selection and will help produce a stable supply of high quality fish.

The part of this work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN).

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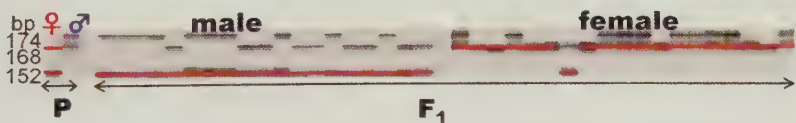


Fig. 6. Electrophoresis image of Sequ21 in linkage group 12 in yellowtail (*Seriola quinqueradiata*). The lower allele (152 bp) from the female parent is a unique allele that was found in the males.

Development and Evaluation of Real-Time Loop-Mediated Isothermal Amplification Methods for the Rapid Detection of Penaeid Viruses

Tohru MEKATA^{*1}, Raja SUDHAKARAN^{*2}, and Toshiaki ITAMI^{*2}

Abstract : Shrimp viral diseases are a major impediment to commercial shrimp farming. The diseases affecting shrimp culture are white spot syndrome virus (WSSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and Taura syndrome virus (TSV). Viral diseases are particularly difficult to control after the onset of infection. Therefore, prophylaxes to prevent or reduce the losses through vertical and horizontal transmission are most important. Various diagnostic methods have been developed to detect shrimp viral diseases, including bioassays, histopathology, polymerase chain reaction (PCR), and quantitative real-time PCR. Although the PCR-based methods are sensitive and highly specific, they require expensive equipment, costly reagents, and are time consuming. Therefore, a simple, quick and sensitive detection method is urgently needed to prevent the invasion of shrimp viral diseases into Japan from other countries. The loop-mediated isothermal amplification (LAMP) assay is a novel approach to amplify nucleic acid with high specificity, sensitivity, and rapidity under isothermal conditions, thereby obviating the need for a thermal cycler. Further, during the LAMP reaction, an insoluble byproduct, magnesium pyrophosphate, is produced in proportion to the large amounts of the target DNA amplified. Hence, real-time quantification can be achieved by measuring the turbidity of the magnesium pyrophosphate using an inexpensive photometer. This real-time LAMP method allows quantitative analysis of nucleic acid templates (real-time LAMP). In the present study, a comparatively less expensive quantitative real-time LAMP assay was successfully applied for detection of shrimp viral diseases and proven to have high sensitivity and specificity.

Key words : RT-LAMP, WSSV, YHV, IHHNV, TSV, Penaeid shrimp, Japan

Introduction

Shrimp viral diseases are a major impediment to commercial shrimp farming. The diseases affecting shrimp culture are white spot syndrome virus (WSSV), yellow head virus (YHV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and Taura syndrome virus (TSV). WSSV was first discovered in northern Taiwan around 1992, and is currently the most serious viral pathogen on shrimp farms throughout the world (Chou *et al.*, 1995; Lo *et al.*, 1996; Flegel, 1997). The

International Committee on Taxonomy of Viruses (ICTV) determined WSSV is the type species of the genus *Whispovirus*, family *Nimaviridae* (Mayo, 2002). The typical clinical signs of WSSV include lethargy, reduced food intake and the appearance of white spots on the carapace (Lightner, 1996). This virus causes 100% mortality within three to 10 days in all life stages of both wild and cultured *Penaeus monodon* and *Marsupenaeus japonicus*, and has a wide host range that includes penaeid shrimp, crabs, copepods and other arthropods (Chen *et al.*, 2000; Syed Musthaq *et al.*, 2006).

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YHV was first reported as a virulent pathogen in the early 1990s causing a 100% crop loss within three to five days post-infection in *P. monodon* in Thailand (Limsuwan, 1991; Chantanachookin *et al.*, 1993). Since then, several researchers have reported the occurrence of YHV in farmed and wild shrimp in Taiwan and many other Asian countries. The causative agent is a rod-shaped, enveloped virus with positive stranded ssRNA containing four open reading frames classified under the genus *Okavirus*, family *Roniviridae*. So far, six genotypes have been identified, and only genotype 1 is known to cause typical signs of YHV infection in shrimp. Other genotypes are widespread in *P. monodon* populations throughout the Indo-Pacific region, but have not been associated with farm disease outbreaks (Wijegoonawardane *et al.*, 2008).

IHHNV is a cosmopolitan virus infecting penaeid shrimp in the Asian-Pacific area and the Americas, but it has not been reported in Japan. The virus causes 90% mortality in *P. stylirostris* juveniles (Lightner *et al.*, 1983) and is detected in other penaeid shrimps (Flegel, 1997). The typical symptoms of the viral disease include reduction in growth and a runt deformity (Lightner *et al.*, 1992; Primavera and Qunitio, 2000). The virions of IHHNV are non-enveloped icosahedrons (22 nm in diameter) containing a single-strand linear DNA of 4.1 kb (Bonami *et al.*, 1990). Genome homology suggests IHHNV may be related to mosquito Brevidensovirus (Shike *et al.*, 2000).

Taura syndrome virus (TSV) is a causative agent of a major disease of the whiteleg shrimp, *Litopenaeus vannamei*. TSV was originally placed in the family *Picornaviridae*, but was later transferred to the *Dicistroviridae*. The complete TSV genome is a linear, positive-sense, single-stranded RNA virus of 10,205 bases. Taura syndrome was first recognized in shrimp farming in Ecuador in mid-1992. The loss was estimated to be close to US\$100 million. Although the TSV originated in Ecuador, it was subsequently discovered in Taiwan in 2000.

Viral diseases are particularly difficult to control after the onset of infection; therefore, prophylaxes to prevent or reduce the losses through vertical and horizontal transmission are most important (Bell and Lightner, 1988; Lotz, 1997). Various diagnostic

methods have been developed to detect shrimp viral diseases, including bioassays, histopathology, dot blot with *in situ* hybridization, polymerase chain reaction (PCR; Lightner and Redman, 1998), and quantitative real-time PCR (Tang and Lightner, 2001).

Although the PCR based methods are sensitive and highly specific, they require expensive equipment, costly reagents, and are time consuming. Therefore, a simple, quick and quantitative detection method is urgently needed to prevent the invasion of shrimp viral diseases into Japan from other countries. The LAMP assay is a novel approach to nucleic acid amplification that amplifies DNA with high specificity, selectivity and rapidity under isothermal conditions, thereby obviating the need for a thermal cycler.

The LAMP assay is based on the principle of autocycling strand displacement DNA synthesis (Notomi *et al.*, 2000). The reaction is performed by DNA polymerase with strand displacement activity and two or three sets of specially designed primers. The assay is highly specific for the target sequence, because that sequence is recognized at six or eight independent sequences. The LAMP method has been used without quantization for diagnosis of various shrimp viruses, such as WSSV (Kono *et al.*, 2004), YHV (Mekata *et al.*, 2006), MrNV & XSV (Pillai *et al.*, 2006), IHHNV (Sun *et al.*, 2006), and TSV (Kiatpathomchai *et al.*, 2007). These qualitative methods cannot, however, determine the copy number of the viral particles present in the sample.

Real-time loop mediated isothermal amplification assay produces large amounts of the target DNA as well as an insoluble by-product, magnesium pyrophosphate, during the reaction, making it possible to perform a real-time measurement of turbidity using an inexpensive photometer (Mori *et al.*, 2001). Real-time LAMP assay has been used for many non-shrimp viruses, such as West Nile virus (Parida *et al.*, 2004), Severe Acute Respiratory Syndrome (SARS) virus (Poon *et al.*, 2005), Dengue virus (Parida *et al.*, 2005) and hepatitis A virus (Yoneyama *et al.*, 2007). In the present study, a comparatively less expensive quantitative real-time RT-LAMP assay was successfully applied for detection of shrimp viral diseases in the field and proven to have high sensitivity and specificity.

Materials and methods

Shrimp: Black tiger shrimp (*P. monodon*) and whiteleg shrimp (*L. vannamei*) with prominent signs of WSSV, YHV, IHNV, and TSV infection, respectively were collected from shrimp farms in Songkhla, Thailand. Shrimp tissue samples were collected in separate sterile tubes and transported to the laboratory on dry ice for the real-time LAMP assay for each viral pathogen.

Nucleic acid extraction: Extraction of DNA was performed from the corresponding viral homogenates prepared from WSSV and IHNV sources. From each sample, 200 μ l of the homogenate were added to 600 μ l of DNAzol reagent (Invitrogen, Carlsbad, California USA) and further steps were carried out according to the manufacturer's instructions. Total RNA was extracted from a pool of heart tissues from the infected shrimp using an RNA extraction kit (High Pure RNA Tissue Kit; Roche Diagnostics, Germany) according to the manufacturer's instructions. Extracted nucleic acid samples were quantified using Nanodrop UV Spectrophotometer ND-100 (NanoDrop Technologies, USA). Synthesis of cDNA for quantitation analysis was carried out

using ReverTra Ace qPCR RT Kit (Toyobo, Japan) with 1 μ g of total RNA as per the manufacturer's instructions.

Design of primers for real-time LAMP procedures: Real-time LAMP primers specific to WSSV, YHV, IHNV and TSV were designed according to published sequences using Primer Explorer Software version 4 (<https://primerexplorer.jp/lamp4.0.0/index.html>, Fujitsu, Japan). The target regions of each shrimp viral pathogen include: ORF36 of WSSV, replicase polyprotein gene of YHV, non-structural protein gene of IHNV, and coat protein gene of TSV (GenBank Accession No. AF369029, EU487200, AF218266 and AF277378, respectively). A set of six or four primers, two outer (F3 and B3), two inner primers (FIP and BIP) and two additional primers (LoopF and LoopB) were designed according to the guideline provided. The oligonucleotide primers used for the amplification are shown in Table 1.

Optimization of reaction temperature: The real-time LAMP was carried out in a total volume of 25 μ l of reaction mixture using Loopamp DNA and RNA Amplification Kit (Eiken Chemical, Japan) according to the manufacturer's instructions for analysis of DNA and RNA viruses. Briefly, the specified

Table 1. Primers used for the LAMP assay for diagnosis of WSSV, YHV, IHNV and TSV

Primer name	Sequences 5' – 3'
WSSV-FIP	TCCGTCTTCAGGGAATACATATGCTCAGGGAAGAAATAGACCATG
WSSV-BIP	GGACCCAAATCGAAATATAAGGCCTATGTTGCCCAAGATCCAC
WSSV-F3	AAACACCGGATGGGCTAA
WSSV-B3	CAAGGCAATACAGAATGCG
WSSV-loopF	GTTAAGAATGATGCATCTAGTGCGA
WSSV-loopB	TGGAACAAAAGATGCTGCTCA
YHV-FIP	CATGTTCCAATTTCCGCCGCTACACACTGAAAATCCTACACG
YHV-BIP	ACCAAGCACTCACCACATTCCTCATGTATAGAGTCAAGT
YHV-F3	CGCTTCTCTCTCTGCTATC
YHV-B3	AAGCATACGTCTCGCATT
YHV-loopF	AGACCGCAGAGAATTTTCCATG
YHV-loopB	CAAAGACATCACATTCACATTCGTC
IHNV-FIP	GAAAACTGGAACAGTTCTTCAGACAAATCAAGACCCTAAACCCAC
IHNV-BIP	ACGAGGAAGACAACCTCTCAAACGTGTATCCACGCAGACCTTAG
IHNV-F3	TCTCCAAGCCTTCTCACC
IHNV-B3	TCCCTCTCGAATTCCCAG
TSV-FIP	AGTTCATCTCAATGCCAGGAAATGAAGACATCAATTATTCGACGC
TSV-BIP	GCAGTCTGAAGCTCGAGCTATTGTTATTACATTTCTGGGGTT
TSV-F3	TGGAATAAGATGAATGCTAAGC
TSV-B3	GACTCAGAACGGAAAGCC

amount of target nucleic acid was mixed with 1 μ l (40 pmol) of each -FIP and -BIP, 1 μ l (5 pmol) of -F3 and -B3 primers, 12.5 μ l of Reaction Mix ($2 \times$), 1 μ l Enzyme Mix containing *Bst* DNA polymerase and distilled water used to make up to 25 μ l. AMV reverse transcriptase was used in the case of YHV and TSV. The reaction temperature (60, 63, and 65°C) was optimized using Loopamp Real Time Turbidimeter LA-200C (Teramecs, Japan). Real-time monitoring was performed every six seconds using spectrophotometric analysis by recording the optical density (OD) at 650 nm. Each assay was carried out three times.

Specificity of real-time LAMP detection: The specificity of the real-time LAMP method was evaluated using different sources of DNA/cDNA templates prepared from WSSV-, YHV-, IHNV- and TSV-infected shrimp and healthy shrimp. Each assay was carried out in duplicate.

Quantitative real-time LAMP: To determine the quantity of unknown nucleic acid using the real-time LAMP assay, the specific target fragments of each viral disease were cloned into plasmids. The amplified PCR product was cloned into pGEM-T Easy Vectors (Promega, USA) according to the manufacturer's instructions. Quantization of the constructed plasmid (using pGEM vector) was achieved using the NanoDrop spectrophotometer, and ten-fold serial dilutions (10^1 - 10^9) were made to evaluate the real-time LAMP assay. The copy numbers of the plasmid DNA were calculated based on the molecular weight and Avogadro's number, and a standard curve was constructed. The standard curve of the specific virus was generated each time during the analysis of samples. The reaction setup was the same as that optimized above, and the reactions were carried out in the Loopamp real-time turbidimeter.

Results

Optimization of real-time LAMP assay conditions

for WSSV, YHV, IHNV & TSV detection: Real-time LAMP assay was performed using DNA/RNA as a template in order to determine the optimal temperature and reaction time for various shrimp viral pathogen. Out of the three different temperatures (60, 63, and 65°C), the best results were obtained at 63°C. The most rapid amplification was achieved at that temperature, requiring less than 20 minutes for the initiation of amplification as determined by a change in the turbidity by magnesium pyrophosphate (Fig. 1). Amplification was efficient at all temperatures tested; however, 63 °C for 60 minutes of reaction time was selected as optimal conditions for further experiments.

Specificity of real-time LAMP detection: Cross-reactivity analysis was performed to examine the specificity of real-time LAMP assay. DNA/cDNA of other shrimp viral disease viruses (WSSV, YHV, IHNV, and TSV) and healthy shrimp were used to determine the specificity of each viral diagnostic assay. As shown in Fig. 2, the real-time LAMP assay was highly specific to each virus without any cross-reaction with other shrimp viral pathogens.

Quantitative detection using real-time LAMP: For quantitative detection of samples of unknown concentrations, a standard curve was generated using the turbidity time (Tt) plotted against the log of the initial template using serially diluted, 10^1 - 10^9 copies/ μ l of plasmids with inserts of concern viral target DNAs (Fig. 3). High correlation coefficient values ($R^2 = 0.99$, $R^2 = 0.99$, $R^2 = 0.98$ and $R^2 = 0.99$, respectively for WSSV, YHV, IHNV, and TSV) were obtained for each viral pathogen by using real-time LAMP assay (Fig. 4). Ten-fold dilutions were used to generate standard curves for each pathogen to run in parallel with unknown samples for quantitative and diagnostic analysis.

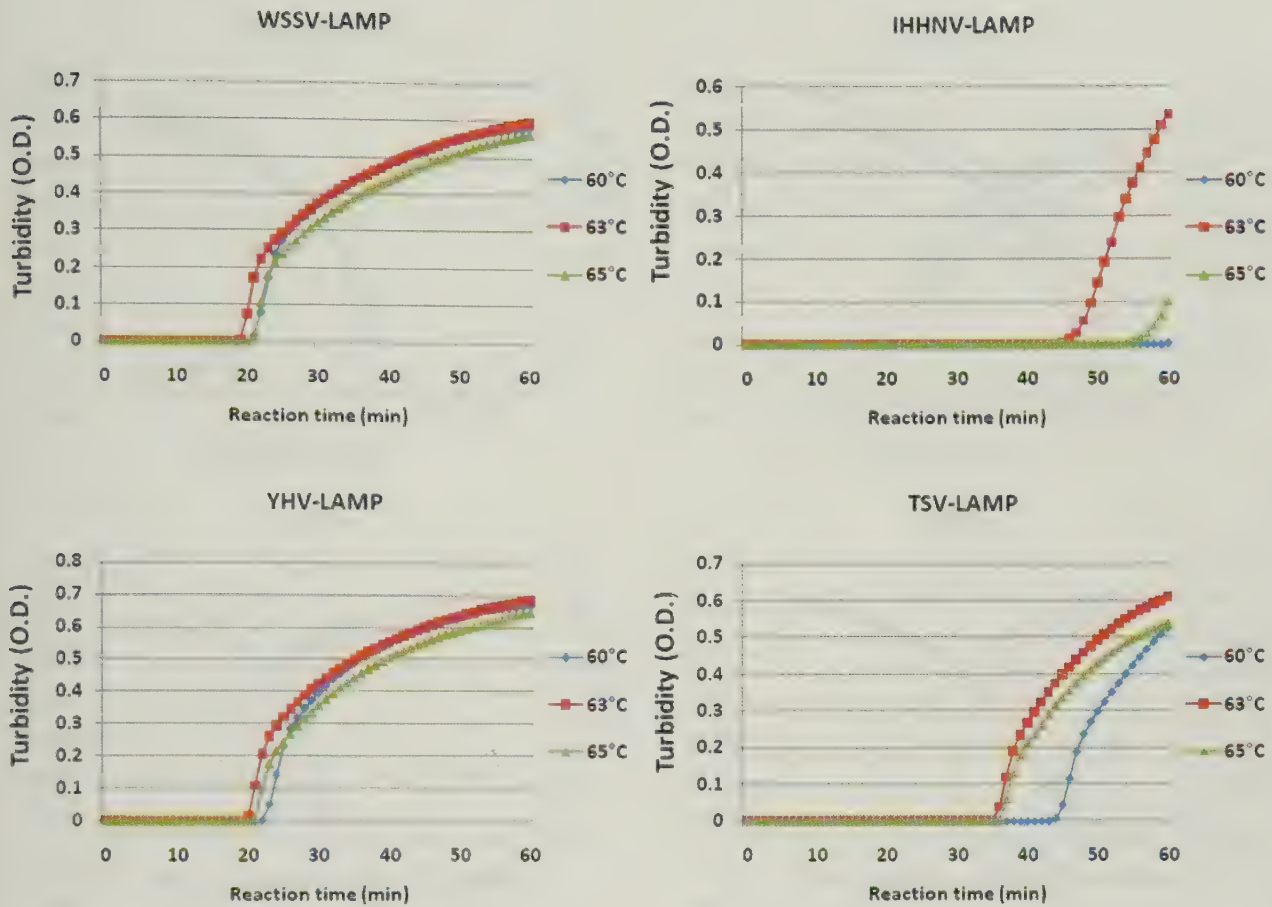


Fig. 1. Optimization of the reaction temperature for real-time LAMP assay of shrimp viral pathogens (WSSV, YHV, IHNV and TSV) performed at 60, 63 and 65°C

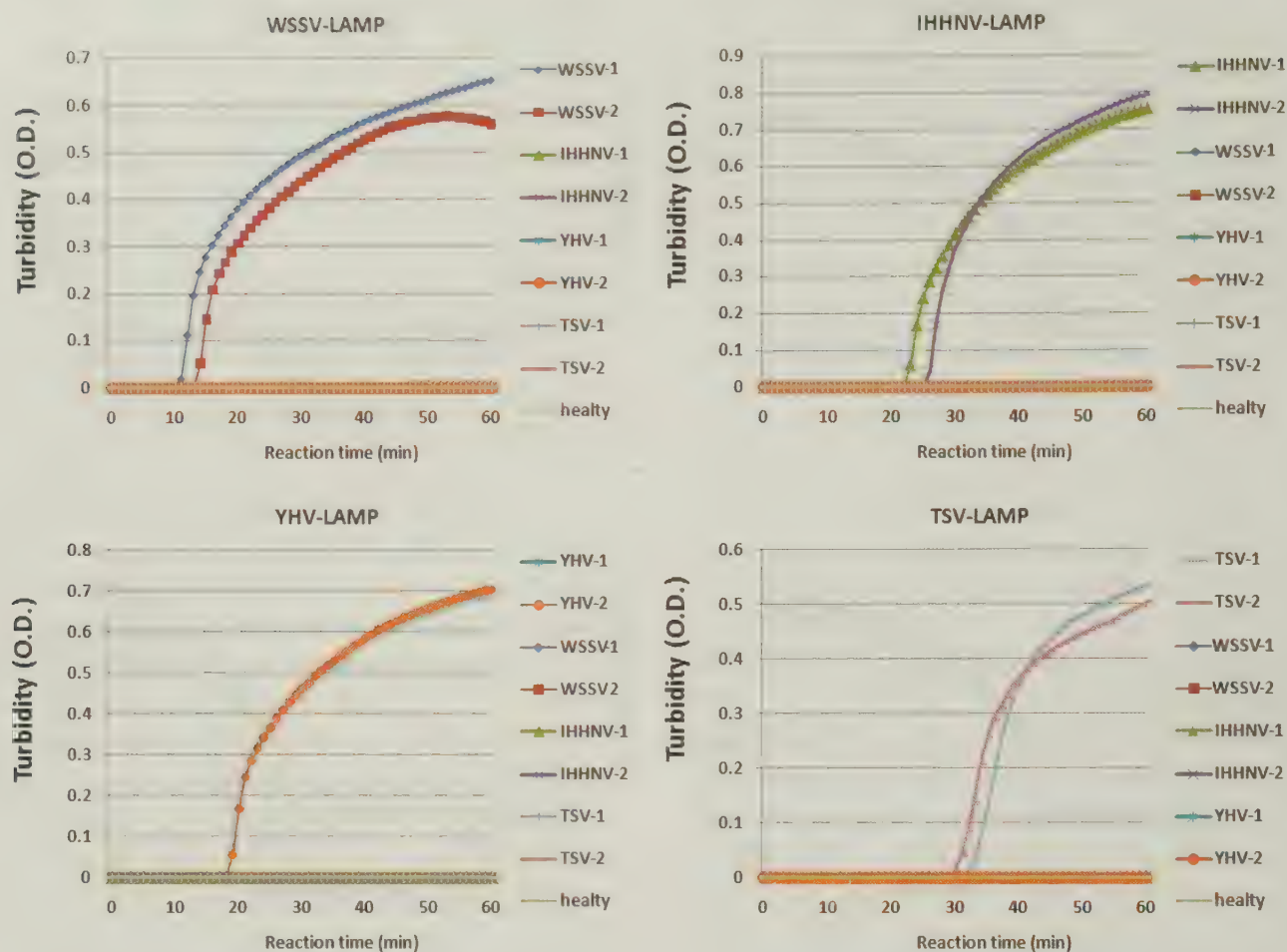


Fig. 2. Specificity of the real-time LAMP assay. Results of cross-reaction analysis with templates of individual shrimp pathogen with other major shrimp viruses (WSSV, YHV, IHNV and TSV- DNA/cDNA, and with a healthy shrimp DNA template are shown).

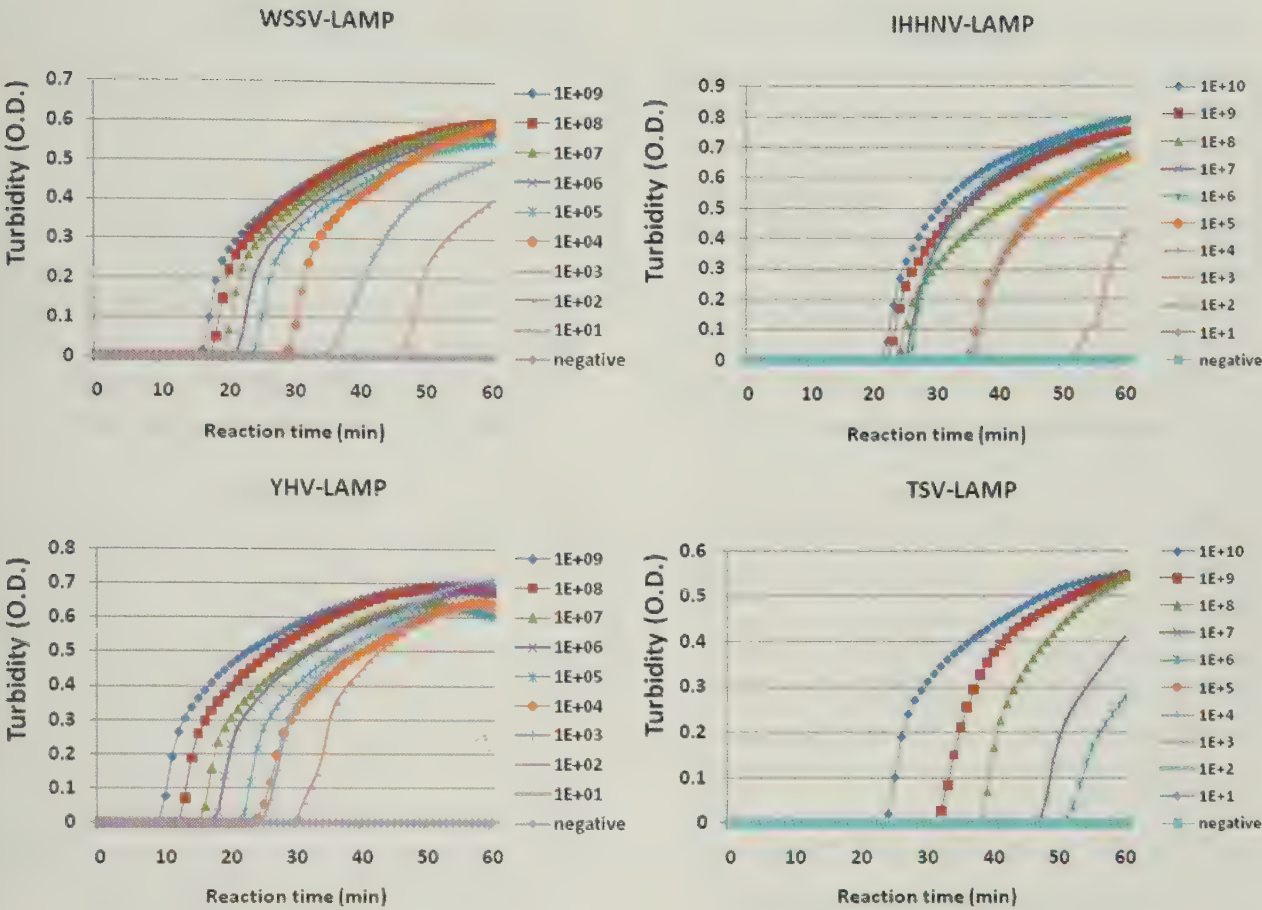


Fig. 3. Real-time amplification of shrimp viral pathogens (WSSV, YHV, IHHNV and TSV) using real-time LAMP assay. Plasmid standards corresponding to target gene of each shrimp viral pathogen at concentrations of 10^1 to 10^9 copies/ μl (time is shown on the X-axis and OD at 650 nm on the Y-axis).

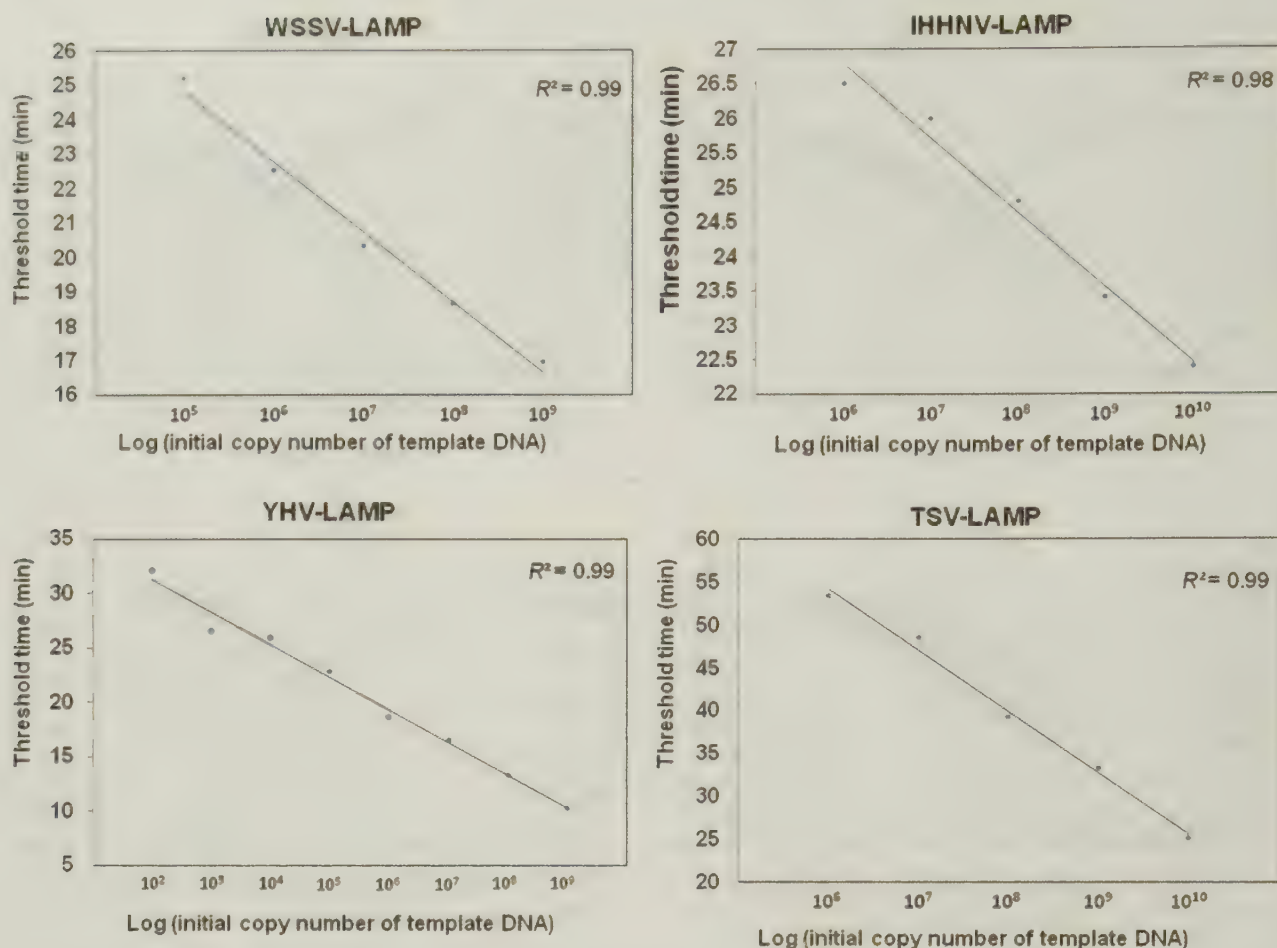


Fig. 4. Standard curves generated from plasmid standards corresponding to target gene of each shrimp viral pathogens.

Discussion

Recent outbreaks of IHNV, YHV, and TSV virus in the Asia-Pacific area and other regions have caused severe economic loss to shrimp farmers; however, these viral disease outbreaks have not been reported in Japan. Therefore, these diseases have been designated as "a specified disease" using Japanese law, where if the disease is detected in Japan, all shrimp must be destroyed to prevent widespread dissemination. Therefore, it is essential to develop an efficient method for field surveillance for these pathogens that has high specificity and sensitivity with a short reaction time, as well as the ability to quantify the viral load. A recent outbreak of YHV in the Asia-Pacific and other regions has

led to severe economic loss to shrimp farmers (Cowley and Walker, 2002) because there was no user-friendly detection assay available.

Here we demonstrate a new diagnostic method for the quantitative detection of shrimp viral pathogens (WSSV, YNV, IHNV, and TSV) using the real-time LAMP method. Early detection of shrimp viral pathogens is important in the shrimp industry for effective health management and preventive measures. Previously, various nucleic acid and protein based assays (Sritunyalucksana *et al.*, 2006) have been employed for the detection of WSSV and other shrimp viral pathogens in the shrimp culture sector. All of these assays can determine only the presence of viral pathogen; however, they do not determine the number of virus particles present.

Quantification of WSSV can be achieved by real-time PCR assays using TaqMan (Sritunyalucksana *et al.*, 2006) and SYBR chemistry (Khadijah *et al.*, 2003, Yuan *et al.*, 2007).

Various conventional diagnostic methods for WSSV has been developed and reported by several researchers worldwide with different sensitivity. Sensitivity of the normal qualitative LAMP assay of WSSV was almost equal to the real-time LAMP assay, whereas it lacks quantitative measurement (Kono *et al.*, 2004). Different PCR-based assays such as one step PCR, Nested PCR, and real-time PCR have the sensitivity limit of up to 1,000 copies, 50 copies and five copies, respectively (Sritunyalucksana *et al.*, 2006). In contrast, the real-time LAMP method is found to be a cost-effective quantitative assay for shrimp viral disease diagnosis. As all conventional methods were developed based on a specific genome, the chance of non-specificity is found to be much less. Real-time PCR assays have been developed for laboratory diagnosis of shrimp viruses; however, these techniques have the intrinsic disadvantage of requiring both high-precision instruments, high costs for the amplification, and a complex method for the detection of amplified products and technically qualified persons.

The increased need for an inexpensive method to quantify shrimp viral pathogens led us to develop the real-time LAMP method. Also the cost of the Loopamp turbidimeter is low, whereas the real-time PCR assays require fluorogenic primers and probes using an expensive fluorometer (Parida *et al.*, 2004). The sensitive real-time LAMP assay has been successfully applied to detect many human pathogenic RNA viruses, resulting in rapid and simple diagnostic measures. The rapid simple detection and quantification of shrimp viral pathogens using real-time LAMP takes less time when compared to other PCR and real-time PCR methods. The optimum conditions for the real-time LAMP reaction were 63°C for 60 min. Higher temperatures can support the rigorous binding of primer and target template in the LAMP reaction than at lower temperatures (Teng *et al.*, 2007), leading to amplicons consisting of concatemer hairpin repeats (Cai *et al.*, 2008). Using the optimized conditions to perform the assay in a short period

of time (<60 min), the turbidity caused by the magnesium pyrophosphate can be visualized without an instrument (Sun *et al.*, 2006). This assay will provide a practical tool in the field for quantitative detection of viral infection in cultured shrimp, even at the early stages.

Each virus-specific standard curve was generated using 10-fold dilutions of 10^1 - 10^9 copies/ μ l of purified plasmids, and the reactions were run in duplicate. The mean Tt for the plasmid standards was generated using the specific software provided with the Loopamp real-time turbidimeter. Standard curve equations were calculated using regression analysis, which compared the average Tt to the standard copy number. We obtained a high correlation coefficient (great than or equal to $R^2 = 0.988$ for each shrimp viral pathogens) for the unknown quantity DNA templates. Cross reactivity analysis showed the primers used were specific to each viral pathogen and did not amplify for other shrimp viral pathogens and healthy shrimp cDNA/DNA templates. Sensitivity analysis showed the assay for each shrimp viral pathogen is detectable up to 100 copies of the template DNA, which is more sensitive than the earlier-developed LAMP and PCR based methods. Furthermore, the gradual decrease of turbidity in the reaction was also clearly observed by the naked eyes.

The real-time LAMP assay is an alternate method to conventional PCR and the LAMP assay (Sun *et al.*, 2006), and in addition, it quantifies the shrimp viral nucleic acid templates. This gives a triplex amplification synchronization on one target gene (Mori *et al.*, 2004). The real-time LAMP assay allows positive samples to become clouded (turbid) and can be viewed visually, eliminating electrophoresis for further confirmation (Mori *et al.*, 2004). The sensitivity of the loop primer was demonstrated in a previous report (Nagamine *et al.*, 2002). The real-time quantitative LAMP assay can be used for gene expression analysis, as the reaction is performed under isothermal conditions and a relatively low temperature where the reverse transcriptase can efficiently work. Thus, this assay has the potential to simplify quantitative gene expression analysis.

We believe the genome specific real-time LAMP assay will be routinely used as a comprehensive

shrimp viral detection system in most field diagnostic laboratories because of its speed, simplicity, specificity and lower cost. We are considering further studies using real-time LAMP with the fluorescence probe, SYBR-I, as the intercalation dye to increase the LAMP sensitivity to quantify very low numbers of virus. The real-time LAMP assay would be a promising technology for shrimp viral pathogen detection, which contributes to better shrimp health management and disease surveillance in shrimp hatcheries and culture ponds for prevention of disease outbreak.

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The Political Economics of United States Marine Aquaculture

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Abstract : Government leasing and regulatory policies are critically important for the development of marine aquaculture. In much of the United States, local, state or national policies constrain the development of marine aquaculture to a scale far below its economic potential. Two extreme examples are the State of Alaska's ban on all finfish farming, and the absence of an enabling regulatory framework for aquaculture in offshore federal waters. This paper suggests five broad reasons for which U.S. policies have been unfavorable towards marine aquaculture: (1) Marine aquaculture is new and small; (2) Fish and marine waters are traditionally public resources; (3) Many Americans perceive potential negative effects of marine aquaculture without offsetting positive effects; (4) NGOs have systematically and effectively opposed marine aquaculture; and (5) The governance system for leasing and regulation is structurally biased against U.S. marine aquaculture. The paper suggests four broad strategies for addressing these political challenges: (1) Fix real problems; (2) Demonstrate benefits; (3) Argue effectively; and (4) Reform governance.

Key Words : Aquaculture, regulation, policies

Introduction

The United States has many potential economic advantages for marine aquaculture. These include a very long coastline, clean water, skilled labor, high levels of technology, excellent infrastructure, a stable legal and economic system, and a large and growing market for seafood. These types of advantages have made the United States a very successful meat producer.

However, United States marine aquaculture production is small and not growing. Why? A critical reason is unfavorable government leasing and regulatory policies. Fish farmers will not invest in marine aquaculture if they can't get leases, or if regulations make aquaculture too costly, or if leasing and regulatory processes take too long, cost too much or are too uncertain and risky (Figures 1 and 2).

Given the importance of government regulatory

and leasing policies, United States marine aquaculture supporters – those who believe that U.S. marine aquaculture can and should grow and that Americans would benefit from it – need to think carefully and clearly about why United States policies are unfavorable toward marine aquaculture, and what they can do to change those policies. This means that they need to think about the political economics of U.S. marine aquaculture: what influences policies, and how policies influence and are influenced by the economics of aquaculture. Marine aquaculture poses significant technical challenges, such as how to design cages, rear juveniles, and increase feed conversion efficiencies. It poses significant economic challenges, such as how to market production effectively and reduce costs. Not surprisingly, aquaculture specialists tend to be trained to address these kinds of challenges and to focus on these kinds of challenges. However, U.S. marine aquaculture supporters need to recognize

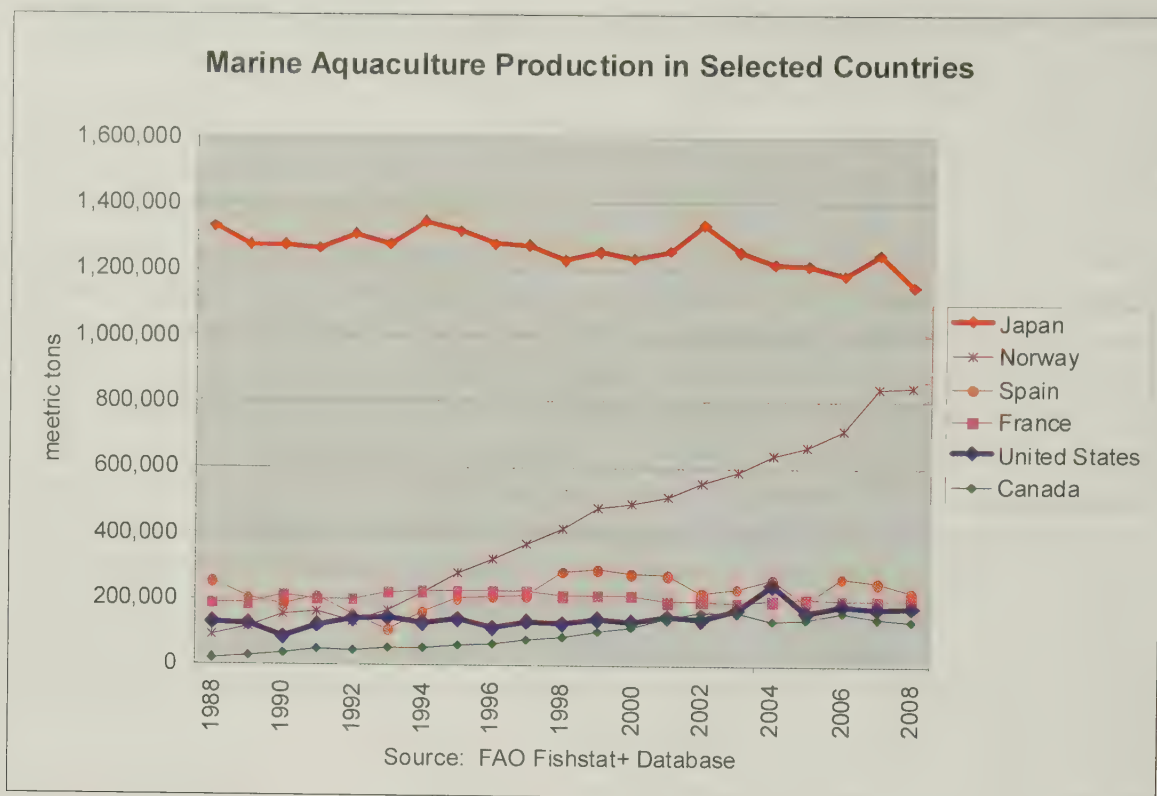


Fig. 1. United States marine aquaculture production is much lower than in Japan or Norway.

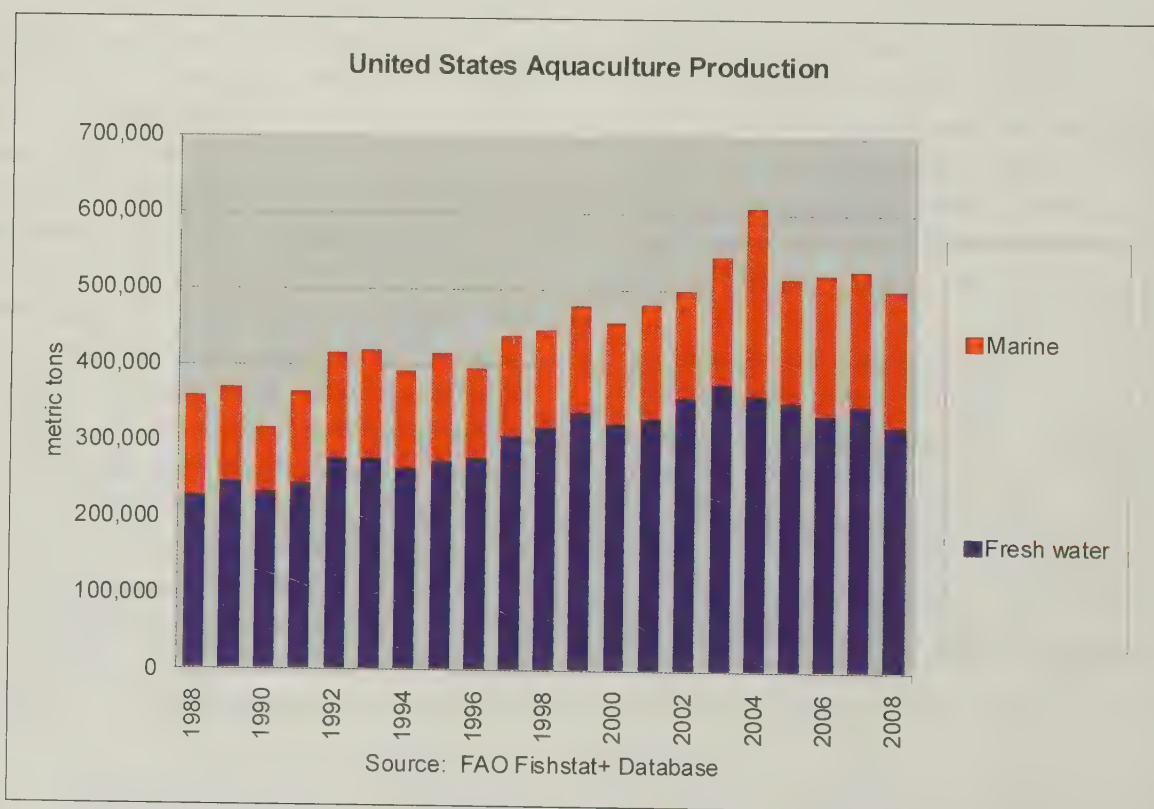


Fig. 2. Most United States aquaculture production is in freshwater.

Table 1. Selected Government Policies Affecting Marine Aquaculture

Types of policies	Selected Key Issues
Leasing policies	Is there a process by which farmers may lease sites? How predictable is the process? How long does it take? How legally secure are sites? How flexible are permitted uses of sites? Can sites be transferred? What do sites cost?
Regulatory policies	What regulations does government impose on farmers? How costly are the regulations? What is the process for developing regulations? How stable and predictable are the regulations? What are the objectives of the regulations? How efficient are the regulations: could the same objectives be achieved at lower cost?
Other policies	How is aquaculture taxed? What kinds of subsidies are available for the aquaculture industry? To what extent and how does government support research, education and marketing? What are trade policies towards farmed fish? What kinds of infrastructure (roads, ports, etc.) does government provide for aquaculture?

that the political challenges faced by U.S. marine aquaculture are as important as the technical and economic challenges. It will require concerted effort to understand and overcome these political challenges in order to achieve leasing and regulatory policies that will enable and encourage responsible development of U.S. marine aquaculture.

Government Policies Affecting Marine Aquaculture

A wide variety of government policies may affect marine aquaculture (Table 1). Conceptually, these may be divided into three broad types: leasing policies, regulatory policies, and other policies. All of these policies matter, but their effects are not symmetrical. Favorable policies such as support for research and marketing cannot offset unfavorable policies such as Alaska’s ban on finfish farming or the absence of an enabling regulatory framework for offshore aquaculture. A single regulatory standard can make farming technically or economically impossible; no single favorable policy can offset this kind of barrier.

It is not just the policies that matter. It is also how stable and predictable they are, and how long it takes to get leases and regulatory approval. Risk

and time are critical to business decisions. *Take the time to get it right, and keep trying to make it better*, might sound like reasonable ways to make public policies, but too much time or too many changes can kill investment that depends on those policies.

Examples of Unfavorable Leasing and Regulatory Policies for U.S. Marine Aquaculture

U.S. marine aquaculture leasing and regulatory policies cannot be characterized in terms of any particular policy of any particular agency. They constitute a very wide range of policies of multiple agencies at federal, state and local levels, which differ widely for different types of marine aquaculture in different states and regions. However, it seems reasonable to conclude that the combined effect of these policies has been to make many kinds of marine aquaculture difficult or impossible in large parts of the United States. Here are some examples: **Alaska finfish farming ban:** Although Alaska accounts for more than half of United States capture fisheries production and more than half of the United States coastline, all finfish farming is banned by the State of Alaska.¹

¹ According to the U.S. Census Bureau’s Statistical Abstract of the United States 2011, Table 360, the “general coastline” of the United States is 12,383 miles, of which Alaska accounted for 6,650 miles (54%). The “tidal shoreline” of the United States is 88,633 miles, of which Alaska accounted for 33,904 miles (38%). According to the National Marine Fisheries Service’s Fisheries of the United States 2009, total U.S. capture fisheries production in 2009 was 3,568 thousand mt, of which fisheries off Alaska accounted for 1,843 thousand mt (52%).

Absence of enabling regulatory mechanism for federal waters: There is no enabling regulatory mechanism for marine aquaculture in federal waters (generally defined as more than three miles offshore). There is no way to apply for or obtain leases to farm fish in federal waters.

Regulatory complexity, inconsistency and delays: Fish farmers face numerous complex, inconsistent, shifting, and time-consuming regulatory requirements. Consider this description by a representative of a major U.S. shellfish farming company based in Washington of challenges faced by the company in obtaining leases:

[One challenge] facing his company and the production of shellfish in the United States is the Army Corps of Engineers' Nationwide Permit 48. Although issued in March 2007, it has yet to be implemented in the Pacific Northwest, and is resulting in inconsistent application in the other shellfish producing states. In addition, there are delays in ESA/MSA consultations and other certification requirements. One of the results of these bureaucratic inactions is that his firm is still waiting – after 15 years – to get a site license in Washington State. These delays have forced the company to purchase leases in Canada, where production has begun and 100 people are employed. Another reason for these delays is that the State of Washington's Shoreline Master Program is being updated. It includes new regulations on the growing of geoducks, a saltwater clam with which [the company] wants to expand its production (United Soybean Board-Aquaculture Industry Coalition, 2011).

In a survey of U.S. molluscan shellfish growers (who account for about two-thirds of U.S. marine aquaculture), Rioux (2011) found that growers perceived significantly higher institutional risks associated with regulation and leasing than risks associated with markets, the environment, or climate. She noted, "through discussions with growers as well as their answers to [an] open ended question, the tie that makes all state and local regulations, regardless of the state or local, the highest risk

is the rate at which they are changed. Growers find that state and local regulations are constantly changing and it is difficult to keep up with them."

According to a review in a recent study of why some aquaculture companies were leaving the United States to invest in other countries, "previous research indicates that strict regulatory environment, cost uncertainties, weak government advocacy, strong local decision-making authority, large number of coastal land owners' opposition, environmental constraints, poor marketing" were factors (Chu, 2009, citing Lockwood, 2001b; Anderson and Bettencourt, 1993; National Research Council, 1992).

Why are United States Policies Unfavorable to Marine Aquaculture?

The starting point in addressing the political challenges to U.S. marine aquaculture has to be clear thinking about why U.S. marine aquaculture faces unfavorable leasing and regulatory policies. Here are five broad contributing factors:

1. Marine aquaculture is new and small.
2. Fish and marine waters are traditionally public resources.
3. Many Americans perceive potential negative effects of marine aquaculture without offsetting positive effects.
4. NGOs have systematically and effectively opposed marine aquaculture.
5. The governance system for leasing and regulation is structurally biased against U.S. marine aquaculture.

1. Marine aquaculture is new and small: Being new and small raises economic challenges for U.S. marine aquaculture. It cannot achieve economies of scale in production, processing, transportation and marketing. It cannot learn and innovate from practical experience.

But being new and small also raises *political* challenges for U.S. marine aquaculture. Because it is new and small, it is harder to demonstrate the benefits and easier to exaggerate the risks of marine aquaculture (Figure 3). As noted by Tiersch and Hargreaves (2002), new resource industries such as

aquaculture face a different political playing field than older resource industries such as logging:

"A core concept of the environmental movement is the precautionary principle, which basically states that it is wise to avoid unnecessary risk... This principle is biased towards slowing or stopping the development of new activities, and shifts the burden of proof from environmental advocates to practitioners such that new activities, like aquaculture, must show that they will not be a problem in the future. This is in contrast to the situation for established industries – detractors must prove that the established industry presents a problem. Of course, newer industries also lack the financial and political resources of groups such as logging, mining and petroleum extraction interests and large chemical corporations. It is easier to restrict or stop aquaculture projects, despite their much smaller environmental risk than it is to attempt to control more damaging established activities. Thus opposing aquaculture development is viewed by advocacy groups as applying an ounce of prevention now instead of the pound of cure that would be required later."

To overcome the political challenges it faces, U.S. marine aquaculture will need committed supporters at all levels of the political and policy process. It will need fish farmers and employees who tell their friends and neighbors and elected officials about the

benefits of aquaculture. It will need supporters who will testify at local public meetings, write letters to the editor, and are elected to local, state, and federal office. It will need organized lobbying efforts to influence state and federal agencies and politicians. All of this takes committed people and money.

Because U.S. marine aquaculture is new and small, relatively few Americans have – or realize they have – a direct stake in it. That means that it has fewer committed supporters, with less money and less political influence. In much of the United States marine aquaculture is still below a political threshold scale necessary for people to understand, accept, and effectively advocate for marine aquaculture. Achieving this scale will be critical to overcoming political challenges. Marine aquaculture will become politically stronger as it grows – but it is difficult for it to grow without being politically stronger.

2. Marine fish and waters are traditionally public resources: The concept of private ownership of land is fully accepted in American law and culture. Although many Americans might think that governments should restrict certain uses of private land, few would argue that private ownership is wrong in principle. Many Americans oppose land-based resource development such as mining or logging or industrial agriculture, but they don't generally base their opposition on the principle that land or resources shouldn't be privately owned.

In contrast, there is no tradition of private



Land farming : traditional and accepted



Sea farming : non-traditional and not accepted

Fig. 3. Two kinds of farming which both affect the environment.

ownership of marine fish or waters in America. Many Americans oppose allowing private exclusive use of or rights to marine coastlines, water or fish. In many cases this principle is firmly set in law. For example, the Alaska Constitution states that, " ... in their natural state, fish, wildlife and waters are reserved to the people for common use."

The tradition that marine fish and waters are public resources imposes an extra political and regulatory hurdle for the development of aquaculture, especially for finfish farming. Before any kind of marine aquaculture can begin, new mechanisms need to be created to allow for exclusive use of marine waters.

Efforts to implement rights-based management regimes for wild fisheries, such as individual fishing quotas, face similar strong philosophical resistance from many Americans. However, as these new management regimes are implemented, public attitudes are likely to shift as the economic logic and advantages of exclusive use rights become more apparent. The same process will likely occur with marine aquaculture – but it will take time.

3. Many Americans perceive potential negative effects of marine aquaculture without offsetting positive effects:

A variety of groups of Americans perceive potential negative effects of marine aquaculture. These include:

- Commercial fishermen, who fear economic competition and environmental damage to wild fish stocks.
- Coastal residents, who fear loss of access to waterfront and changes in the views they enjoy.
- Environmentalists, who worry variously that marine aquaculture will cause pollution, harm marine ecosystems, or increase pressure on global wild fish stocks harvested for production

of fishmeal and fish oil used in fish feeds.

These groups play significant roles in the politics of United States marine aquaculture, across the political and regulatory process at local, state, and national levels. For example, Alaska salmon fishermen spearheaded the Alaska legislature's 1990 ban on finfish farming, and continue to vocally oppose aquaculture development in federal waters nationwide, along with Alaska's congressional delegation (Figure 4).

Similarly, coastal residents have strongly and effectively opposed marine aquaculture in states such as Maine and Washington. Sebastian Belle, Executive Director of the Maine Aquaculture Association, described the political challenges facing aquaculture as a result of demographic shifts in coastal regions:

"In Maine...part of the application process for the series of permits and licenses needed to operate in the marine environment is an exhaustive series of meetings with the general public and all stakeholders. Part of the constituency will not like what you do, whatever you do. [Because of] a demographic shift to a population-base of retirees from other states, as summer-home visitors to our beautiful coast became year-round residents, ...coastal communities now view the ocean for 'recreational use,' and commercial fishermen and aquaculturists must make their case locally to people who have no history or link with the ocean for making a living" (Thomas, 2011).

These groups' opposition is vexing and frustrating to marine aquaculture supporters who feel that the objections and fears of aquaculture opponents are exaggerated, unfounded, or simply irrational. How do you argue with people who – without any scientific basis – believe that marine aquaculture will destroy



Fig. 4. Alaska bumper sticker.

commercial fisheries? How do you argue with people who claim that fish farms that will be barely visible will destroy their coastal view? How do you argue with people who appear to be unwilling to accept any level of risk or change?

The political reality is that it is rational for groups which perceive only negative potential effects of marine aquaculture to oppose it. Why accept any risk if there is nothing to be gained?

Clearly there are many things to be gained from marine aquaculture; such as stable jobs, tax revenues, and synergies with other marine industries including commercial fishing, good food, and a reduction in import dependence. But, in many areas, aquaculture supporters have failed to make the case effectively that aquaculture has these positive potential benefits.

4. NGO's have systematically and effectively opposed U.S. marine aquaculture: Numerous U.S. Non-Governmental Organizations (NGOs) have invested significant funding and effort to advocate banning, delaying, restricting, or regulating U.S. marine aquaculture in ways that increase the risks and costs of investment. Collectively these organizations have played a major role in influencing the public, the press, politicians, and regulators in ways which have contributed to unfavorable leasing and regulatory policies towards marine aquaculture.

NGOs that have funded or engaged in significant advocacy to influence U.S. marine aquaculture policies include the Packard Foundation, the Moore Foundation, the Pew Charitable Trusts, Greenpeace, the Environmental Defense Fund, Food and Water Watch, and others, both large and small. The scale, objectives, strategies, and arguments of these groups vary widely, making it difficult to generalize about their motives, methods, and effects. All of these organizations would assert that they use rational and science-based arguments to advocate for the public interest. Marine aquaculture supporters would argue that the NGOs engaged in aquaculture advocacy range from responsible to grossly irresponsible and that they pursue strategies ranging from ethical to grossly unethical.

As noted by Tiersch and Hargreaves (2002), "Advocacy groups can provide a valuable service

by acting as an impartial watchdog of environmental issues and calling attention to legitimate concerns." However, a very real and frustrating challenge for marine aquaculture supporters is that some NGOs appear willing to say anything to oppose marine aquaculture, with casual and sometimes blatant disregard for objectivity, truth, or the complex reality of what experience and science have shown about the hugely varied effects of the hugely varied kinds of activities collectively known as aquaculture. Here, for example is a statement posted on the website of the *NGO Food and Water Watch*:

"Many fish-lovers would be horrified to learn that huge quantities of fish and shrimp are now being grown in giant nets, cages, and ponds where antibiotics, hormones and pesticides mingle with disease and waste. These industrialized aquaculture facilities are rapidly replacing natural methods of fishing that have been used to catch fresh, wild seafood for millennia."

It is difficult for people in industry, government or science to refute these kinds of arguments when they are held to much higher standards of argument and evidence.

Amplifying the efforts of NGO aquaculture advocacy are articles in the popular and increasingly in the so-called 'scientific' press. Tiersch and Hargreaves (2002) characterized this relationship as follows:

"Much of the criticism of aquaculture by NGOs began as opinion pieces in news media or as information provided by specific advocacy groups. Gradually this material began entering scientific literature as news items and recently has shifted into the arena of scientific review and technical articles, and special reports for commissions. In effect, NGOs have become clearinghouses for information critical of aquaculture. Various groups have adopted attacks through popular media as a method to bring about changes in popular opinion and regulatory policy. This approach is not discouraged by the media because sensational accusations, controversy and polarized debate are considered to be newsworthy simply for their mass appeal rather than scientific validity."

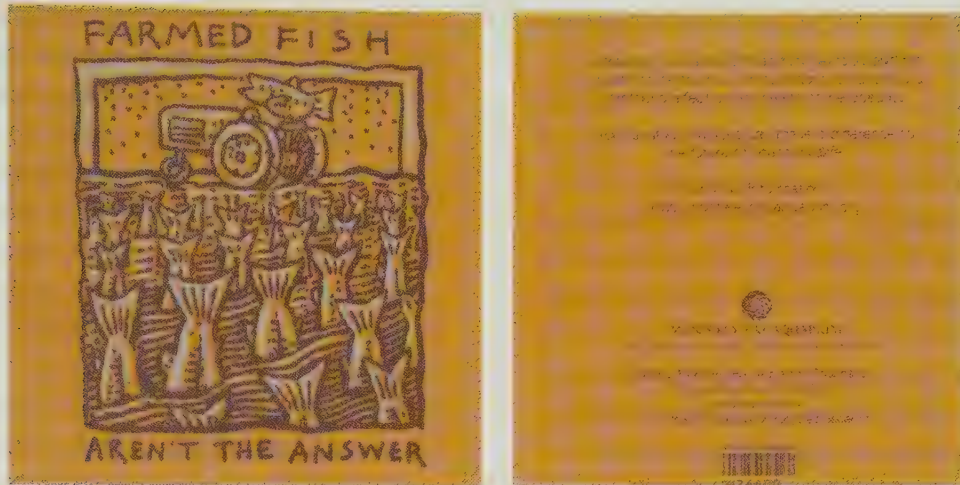


Fig. 6. A greeting card sold by the Monterey Bay Aquarium.

5. The governance system for leasing and regulation is structurally biased against U.S. marine aquaculture:

The governance system for U.S. marine aquaculture leasing and regulation consists of the processes by which leasing and regulatory policies are developed by the agencies that have authority to develop policies, and how they make those policies. For purposes of discussion, we may define a hypothetical unbiased governance system as one that would develop policies based on an objective consideration of the best interests and/or preferences of society as a whole, balancing both costs and benefits. For several structural reasons, the U.S. governance system is likely to be less favorable toward aquaculture than an unbiased governance system would be.

One reason is that leasing and regulatory authority for U.S. marine aquaculture is fragmented among multiple branches of government (executive, legislative, and judicial) at multiple levels of jurisdiction (local, state, and federal agencies). Federal agencies with leasing or regulatory authority for marine aquaculture include, but are not limited to the National Marine Fisheries Service, the Army Corps of Engineers, the Environmental Protection Agency, the Fish and Wildlife Service, the Department of Agriculture, and the Food and Drug Administration. Similarly, at the state level, environmental and fisheries agencies typically have regulatory authority. Local governments may exercise additional authority, such as zoning

regulations. In the legislative branch, the U.S. Congress and state legislatures enact laws affecting aquaculture, and many issues are decided by the courts at both the state and federal levels.

Several structural biases against aquaculture result from this fragmented governance system. One bias is that most agencies have a limited focus. Rather than considering the best interests and/or preferences of society as a whole, or balancing both costs and benefits of marine aquaculture, they are charged with more narrow and specific goals, such as protecting water quality or promoting economic development. Even though some agencies may be charged with considering the benefits of marine aquaculture, this does not result in an unbiased governance system. A single agency – at any level – can stop marine aquaculture even if all other agencies are willing or eager to promote it. For example, if a single agency establishes impossible water quality regulations or simply takes too long to decide what the regulations will be, it can stop or indefinitely delay aquaculture investments.

A second structural bias is that agencies may be biased internally against aquaculture. For example, fisheries management agencies may be strongly influenced by constituents who oppose aquaculture, such as fishermen. Their staff may have little interest in or knowledge of aquaculture, or may actively oppose it. This is particularly likely to be the case because aquaculture is new and small, so regulatory jurisdiction is typically within agencies

established to regulate and promote older and larger industries.

A third structural bias is that agency budgets for aquaculture leasing and regulation are limited. When budgets are limited, agencies do less. But U.S. marine aquaculture can only develop if agencies are proactive in developing enabling leasing and regulatory frameworks. Doing less will delay the development of marine aquaculture.

Aquaculture advocates often argue that leasing and regulatory policies should be driven by science and reflect an objective consideration of economic and environmental costs and benefits. However, it is uncertain whether a science-driven and objective governance process is possible for marine aquaculture. In a recent thoughtful essay about the challenges facing Canadian aquaculture, Rayner (2008) argued that it isn't:

"Aquaculture presents special problems of policy coordination that urgently require a more collaborative, less "top-down" approach to policy-making than traditional governing arrangements are able to deliver. There has been a general loss of confidence in authoritative coordination as the basis for public policy. Not only are citizens less inclined to accept the decisions of their territorially-based governments about aquaculture development as definitive (the legitimacy problem), they are even less likely to be impressed by the knowledge that the decision has been informed by the advice of a group of self-accrediting experts – which is, of course, why senior public servants and ministers are not especially eager to seek out this advice in the first instance.

"The reversal of political relationships has many important consequences. [One] is the phenomenon that so baffles and enrages those involved in resource industries: they can take part in all kinds of time-consuming planning exercises and comply with every legal requirement in the jurisdiction in which they are operating but still be targeted by activists who challenge the legitimacy of the original plan or regulation. The response of Canadian aquaculture to these developments can neither be "trust us, we know what we are doing," as

the scientific establishment is inclined to say, nor "wait until all the facts are in," as the more radical exponents of the precautionary principle are asserting. Trust has gone and all the facts will never be in (which, of course, fits happily with the political agenda of those making the latter claim). The question of action, of what to do, and more specifically, of what we as a 'community of interest' in aquaculture can do, remains."

Political Strategies for U.S. Marine Aquaculture

What can the community of interest in U.S. marine aquaculture do? What are political strategies that can overcome the political challenges faced by U.S. marine aquaculture? This question is being raised, with increasing urgency, within the industry and among supporters in government, science, and the broader public.

Below, I briefly discuss four broad strategies:

1. Fix real problems
2. Demonstrate benefits
3. Argue effectively
4. Reform governance

Although their relative importance will vary for different types of marine aquaculture and in different regions, all four strategies will be necessary for U.S. marine aquaculture to achieve its full economic potential.

1. Fix real problems: Where there are real problems associated with marine aquaculture – such as escapes, disease or pollution – the industry needs to address them. Public opinion and policy will not support marine aquaculture where there are substantive reasons for not supporting it. Fairly or unfairly, all segments of marine aquaculture have a stake in addressing problems anywhere within the industry. As with other resource industries (including commercial fishing) problems anywhere in the industry have the potential to affect broad perceptions of the entire industry and policies affecting the entire industry.

2. Demonstrate benefits: It will not be enough for marine aquaculture to demonstrate that it does no

harm. Some groups will never be convinced, and doing no harm will not generate committed support. Gaining committed support will require making the case effectively that aquaculture offers significant potential benefits at the local, state, and national levels, including benefits for groups that have tended to oppose aquaculture, such as processing synergies and marine jobs for commercial fishermen, and tax revenues for coastal residents.

3. Argue effectively: To overcome significant, well-funded and sometimes unscrupulous opposition, U.S. marine aquaculture supporters need to argue their case much more effectively than they have in the past. They need to communicate more effectively with the public, the press, politicians, and regulators. They need to more effectively understand and counter the arguments and tactics of anti-aquaculture advocacy groups at local, state, national, and international levels. They have been trying, but they need to try harder, and more effectively, and with more resources and coordination. How to do this is the subject of much discussion within the industry and among its supporters.

In a thoughtful article, Tiersch and Hargreaves (2002) offered a number of practical and well-reasoned suggestions for responding to criticism by advocacy groups:

- Respond from the perspective that criticisms and solutions must be based on a comprehensive and balanced view of the total problem.
- Respond to criticism with clearly presented, broad-based arguments.
- In responding to criticism, recognize that information can be used to achieve different ends.
- Refer to specific sectors rather than reinforcing the misconception of the existence of a collective aquaculture industry that is operated, regulated, and culpable as a single entity.
- Be familiar with the role of aquaculture in economic development, especially in developing countries.
- Know your critics, their methods, and their goals.
- Recognize legitimate criticism.

- Do not shift blame to other sectors of aquaculture to deflect legitimate criticism.
- Learn how the media can be used as a conduit for responses to criticism.

In a recent presentation, Sebastian Belle, Executive Director of the Maine Aquaculture Association, offered a wealth of practical advice gained from years of practical experience:

“Over the last 20 years, we’ve learned that it takes basic common sense, hard work, and a lot of time to win the social license to operate. You’ll never get 100% acceptance, but if you can get locals to feel that it is “their” neighborhood farm, by sharing holiday seafood, becoming a part of their lives, helping them to be familiar with operations, they can change their attitudes. It doesn’t happen with outside lawyers or environmental groups who come to town for their own agenda, with no vested interest in finding solutions. We talk directly to the people who are local and close to us, and avoid gatekeepers and external stakeholders. You’re only as good as your last failure, so admit your mistakes and learn from them. Get to know the community and your audience, and talk to them. The best thing is to be good at listening to people. All concerns are legitimate by definition. Listen to every one of them, respond to every one of them. Always follow through. Never mislead or be evasive. Be polite. Avoid being defensive. Form strategic partnerships. Communicate, use visual aids, show what a farm looks like to dispel fear of the unknown. Do your homework: find out what to do to make the community, the locals, comfortable with aquaculture.” (Thomas, 2011).

4. Reform governance: Ultimately, the political challenges to U.S. marine aquaculture cannot be overcome by arguing more effectively. It will also require reforming governance so that leasing and regulatory policies are based on a consideration of both costs and benefits, and accommodate the legitimate interests and concerns of farmers, environmentalists, coastal residents, and other stakeholders. Countries such as Norway have succeeded in doing this. U.S. aquaculture advocates

need to learn more about how they have done so, and to give thoughtful consideration to new forms of governance based less on confrontation.

Rayner (2008) suggested that reform of aquaculture governance should include "the creation of more sophisticated aquaculture policy networks, more use of tools of self-regulation, and more open coordination and benchmarking." Despite the recognized challenges for the aquaculture industry of working with critics, Tiersch and Hargreaves (2002) offered the thoughtful observation that this approach may ultimately prove most successful:

"Realistically, the best approach to dealing with advocacy groups is to devote effort in gaining a strong personal understanding of the relevant issues, and to be proactive in addressing problems and communicating solutions. An increased awareness of social, economic, ecological and political issues will allow those involved in aquaculture to be proactive and avoid taking a defensive, reactionary position. Indeed, it is likely that aquaculturalists and environmental advocates share values at the heart of most issues, and it is the tactics used in addressing the inappropriate actions of a minority within aquaculture and environmental advocacy that drive groups apart."

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Inland Marine Fish Culture in Low Salinity Recirculating Aquaculture Systems

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Abstract : Expansion of marine aquaculture is challenged by the high cost and limited availability of coastal land and water resources, effluent concerns, high production costs, restricted growing seasons, lack of quality fingerlings, and inadequate regulatory and permitting processes. Many of these constraints can be addressed with inland marine fish culture in low salinity recirculating systems as production models. We describe recent and ongoing development of technologies in four principal areas: 1) engineering and system design; 2) year-round fingerling production; 3) diet development; and 4) physiological adaptation of marine fish to low salinity environments using genomic approaches. It is anticipated these technologies could find application for rearing euryhaline marine fish throughout approximately 2/3 of the U.S. where low salinity groundwater is available. This approach will reduce the need to be located near the coast, reduce the volume of saltwater effluent, and reduce the carbon footprint of marine finfish production.

Keywords: low salinity, marine fish, recirculating systems

Introduction

A growing and increasingly health-conscious population, coupled with declining capture fisheries, is driving increased global demand for farm-raised seafood that can only be met through expansion of aquaculture (Delgado *et al.*, 2003). In 2007, aquaculture represented 33% of total global seafood production and is projected to increase to as much as 71% by 2030. The U.S. aquaculture industry represents a US\$ 1 billion/year industry; however, the U.S. still imports 84% of its edible seafood resulting in a US\$ 9 billion trade deficit (NOAA, 2007). The U.S. aquaculture industry is principally based on production of freshwater finfish, with salmon representing only 5% of the industry

being the only notable exception. This suggests tremendous potential for growth in a developing U.S. marine aquaculture industry. However, development and expansion of marine aquaculture is challenged by the high cost and limited availability of coastal land and water resources, effluent concerns, high production costs, restricted growing seasons, lack of quality fingerlings, and inadequate regulatory and permitting processes (FAO, 2009). Many of these constraints can be addressed with inland marine fish culture in low salinity recirculating aquaculture systems (RAS) as the production model. We describe recent and ongoing development of technologies for this production model in four principal areas: 1) engineering and system design; 2) year-round production of fingerlings; 3) diet development;

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and 4) physiological adaptation of marine fish to low salinity environments. It is anticipated these technologies could find application for rearing euryhaline marine fish throughout approximately 2/3 of the contiguous United States (Fig.1) where lightly saline groundwater is available (Alley, 2007). We have worked with several species, including southern flounder (*Paralichthys lethostigma*), summer flounder (*Paralichthys dentatus*), hybrid striped bass (*Morone chrysops* × *M. saxatilis*), black sea bass (*Centropristis striata*), red drum (*Sciaenops ocellatus*), and cobia (*Rachycentron canadum*), but this paper is focused principally on our work with Florida pompano (*Trachinotus carolinus*), the project's model species.

Engineering and System Design

Although RAS performance for freshwater production has been well characterized, less information is available for saltwater RAS production. Saltwater RAS present several challenges, including: 1) saline water holds less dissolved oxygen (DO) than

freshwater; 2) lower oxygen transfer and nitrification rates; 3) solids are more buoyant and difficult to remove; and 4) carbon dioxide (CO₂) is more difficult to measure using standard methods (Pfeiffer *et al.*, 2010).

Traditional RAS utilize high pressure centrifugal pumps to move water for filtration, aeration, degassing, and delivery to culture tanks. However, centrifugal pumps can account for more than 50% of the system's energy consumption as their operating efficiency is typically about 80% (Pfeiffer and Wills, 2009). An alternative is to utilize axial flow propeller pumps and airlift technology to move large volumes of water under low pressure. Those technologies have been found to reduce energy use by approximately 33%.

Conventional gas transfer technologies require a large amount of space, capital investment, and contribute to energy demand. In addition, diffused aeration in a circular tank can interfere with the hydrodynamics of water rotation and efficiency of solids removal. We evaluated low-head systems utilizing airlift technology for water movement,

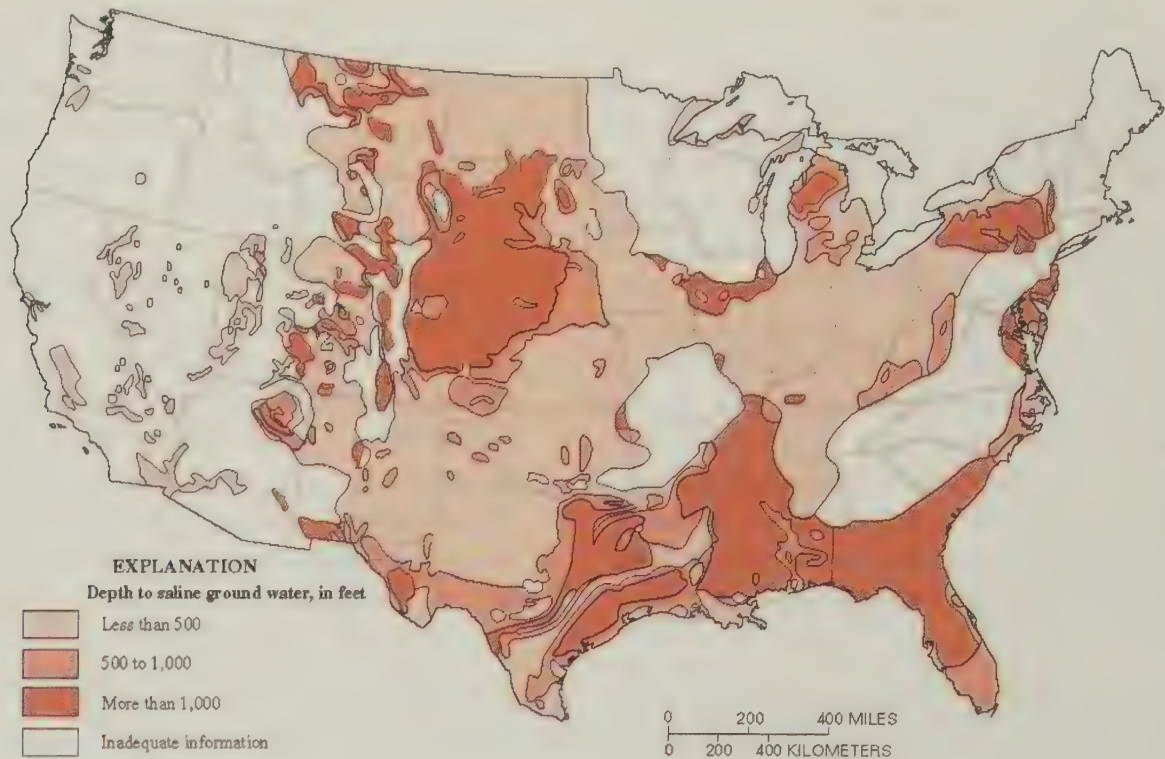


Fig. 1. Preliminary perspective on the location and depth to reach saline groundwater in the contiguous United States (adapted from Alley (2007) as generalized from Feth and others).

aeration, and degassing. Gas:liquid ratios, lift:submergence ratios, and flow rates in the airlift systems were also evaluated for their effect on gas transfer rates. Oxygen mass transfer rates ranged from 0.01 to 0.09 kg O₂ hr⁻¹ based on airlift pipe diameter, water flow rate, air-flow, and dynamic head. A modification of the airlift design to give higher water flow at low dynamic head improved oxygen addition and CO₂ removal, providing 0.54 mg l⁻¹ of O₂ to the water and removing 1.6 mg l⁻¹ of CO₂ per pass.

In moving and static fixed film biofilters the ammonia removal rate varies with feed loading, salinity, and hydraulic flow rates. Ammonia removal rates ranged from 50 to greater than 1,200 g TAN m⁻³ media day⁻¹ in static submerged fixed film biofilters using standard floating plastic media and removal rates correlated with influent TAN concentrations. With increasing salinity the nitrification rates decreased approximately 30%. When plastic media offering greater protective surface area was compared with the standard plastic media, significant differences in TAN removal rates were observed once feed loading exceeded 0.4 kg feed m⁻³ media. The range of TAN removal in a 0.71 m⁻³ moving bead biofilter ranged from 20 to 250 g TAN m⁻³ media day⁻¹. In airlift moving bed torrus filters with kaldness media (0.37 m³) TAN removal rates were under 300 g TAN m⁻³ media day⁻¹. Increased TAN removal efficiency from 36 g to 180 g TAN removed per dollar spent was realized. Increases in nitrification rates at higher feed loads allow biofilters to support a greater biomass of fish and improve production costs.

Other recent accomplishments include the evaluation of individual system water treatment components and development of design criteria. Ammonia removal efficiency of two types of filters, efficacy of TAN removal with different substrates, and solids removal efficiency of three additional system components were determined. Ammonia removal rates and performance curves for a fixed film biofilter were established over a wide range of salinities, providing models to design filtration devices for specific salinity needs.

A swirl separator, static bed filter, and moving bed torrus filter were evaluated for suspended solids

removal during low salinity culture of red drum. Total suspended solids analyses were conducted to determine the particle size distribution and percent removal efficiency by each filter type. The static bed clarifier removed 23% of particles $\leq 55 \mu\text{m}$. Overall, the static bed exhibited the best removal efficiency of particles of all size ranges and was the most efficient clarifier.

To minimize labor costs associated with filter maintenance, foam fractionation technology was also evaluated for its ability to remove suspended solids under low salinity conditions (11-13 ppt). Efficiency of a foam fractionator was analyzed using different methods and design criteria including microbubble generation, ozonation, and hydrodynamic head. Results indicated greater particle removal with the use of ozone and at a higher operating water height.

These advances and component optimizations have been implemented in a research scale production facility with four replicated 45 m³ high-head and four replicated 43 m³ low-head RAS, each with four 3.05 m diameter tanks, rotary microscreen drum filters, biofiltration, supplemental oxygenation, ultraviolet light sterilization, degassing, and water and energy monitoring capabilities. The tanks have automatic feeders and oxygen sensors outfitted with programmable logic controllers (PLC) for feed delivery and controlled oxygen input.

In intensive RAS the use of supplemental oxygen increases carrying capacity of the system. Liquid oxygen can reduce unit production costs through production of greater biomass per unit volume. However, since saline water holds less DO at saturation than freshwater, the margin between DO saturation and deficiency is much narrower, which can result in wasteful overuse of oxygen, reduced production, or mortalities. Utilization of PLC maintains the percent saturation levels in the tanks between designated set points. Implementation of PLC technology for individual tank oxygen control has reduced daily oxygen to one-fifth of that in uncontrolled systems.

The production facility has been used for experimental trials with hybrid striped bass (Rawles *et al.*, 2006), Florida pompano (Weirich *et al.*, 2009; Pfeiffer and Riche, in press) and cobia (Weirich *et al.*, 2010) reared to market size. This was the first

demonstration that pompano and cobia could attain market size utilizing RAS technologies. Furthermore, it was demonstrated that pompano could reach market size under low salinity conditions (5 g l⁻¹). Future research activities include: 1) developing nitrification and aeration curves for a moving bed biofilter with different volumes of media evaluated at various feed loading rates in a low-head RAS under low salinity conditions; 2) determining the solids removal efficiency and relative cost to benefit of implementing foam fractionation technology to improve removal of fine and suspended solids; and 3) determining solids removal efficiency of various microscreen mesh sizes on a rotary drum filter in a low-head RAS. We also propose to design a sustainable effluent treatment system to recover effluent wastewater for reuse as rinse water on microscreen drum filters, and convert the sludge to a salable secondary by-product. In addition, we are exploring alternative means for addressing off-flavor problems that can arise during final production in RAS.

Year-round Production of Fingerlings

Lack of techniques for sustained production of fingerlings is a principal bottleneck for marine fish production. Therefore, a domestication program for Florida pompano was developed. Protocols for the capture, quarantine, feed training, conditioning, and spawning induction were established and described elsewhere (Weirich and Riley, 2007). During conditioning and spawning, Florida pompano were fed a modified commercial gelatin-based broodstock diet formulated for pompano (Gelly Belly, Florida Aqua Farms, Inc., Dade City, FL). Volitional spawning was induced via administration of a gonadotropin releasing hormone analogue (GnRH-a). In 2004 and 2005 a total of nine spawning trials were conducted with mixed but generally increasing success (Table 1). Since then, methods for rearing pompano larvae using RAS have been established, with post-larval transformation occurring at 17-days after hatch (DAH) and survival rates as high as 50% from egg to metamorphosed juvenile (Weirich *et al.*, 2005; Weirich, 2009). Recently we were able to attain year-round spawning, with one of six populations

of females spawning as many as 10 consecutive months, and three others at least 7 months with no diminishment of egg quality.

Larval and juvenile development have been characterized for pompano, black sea bass, and southern flounder via imaging analysis to document ontogenetic development of early life stages. Events such as yolk absorption rate, first feeding, and flexion as well as monitoring of growth, mouth development, and metamorphosis in Florida pompano have been reported elsewhere (Riley *et al.*, 2009). Mouth gape and other rates of development are utilized to appropriately size live feeds and microparticulate diets. Additionally, the tolerance of pompano eggs and larvae to ammonia and nitrite, as well as their sensitivity to salinity and copper have been determined. As RAS are prone to buildup of the metabolites ammonia and nitrite, and toxicity is dependent on salinity and other water chemistry parameters we have determined ammonia and nitrite toxicity for post-metamorphic juveniles at various salinities (Weirich and Riche, 2006).

Optimal feeding protocols for pompano larvae have been established (Riley *et al.*, 2009). Efforts to improve live feed production and nutritional value have been addressed. Rotifer and *Artemia* enrichment protocols were established, and nutritional value of enrichment products evaluated via growth, survival, and fatty acid analyses (Cavalin and Weirich, 2009). Due to the small mouth gape of pompano at first feeding, evaluation of the copepod *Pseudodiaptomus pelagicus* was also undertaken (Cassiano *et al.*, 2011). Co-feeding and weaning strategies were developed and results indicate that pompano can be weaned to dry diets without compromising growth and survival beginning as early as 14 DAH.

Future research activities include evaluating alternative strategies to reduce time to weaning with the goal of eliminating *Artemia* from the larval production cycle. Additionally, we propose to evaluate commercial larval diets and develop novel microparticulate diets to meet the nutritional requirements of pompano larvae.

Table 1. Initial attempts at volitional spawning of Florida pompano following GnRH-a administration (data adapted from Weirich and Riley 2007)

Spawning Event	Number Females	Eggs Collected	Number Floating	Number Sinking	% Fertilization (total eggs)	% Fertilization (floating eggs)	% Hatch (fertilized eggs)	Eggs per Female
<u>Year 2004</u>								
1	8	2,786,000	459,000	2,327,000	14.3	86.8	73.1	348,000
2	4	921,000	368,000	553,000	28.5	71.3	76.5	230,000
3	6	736,000	152,000	584,000	18.0	87.3	79.7	123,000
<u>Year 2005</u>								
1	3	1,156,000	428,000	728,000	36.7	99.0	88.2	385,000
2	3	454,000	21,000	433,000	4.5	96.9	79.4	151,000
3	3	452,000	322,000	130,000	63.7	89.4	83.8	151,000
4	4	753,000	278,000	475,000	36.7	99.3	80.7	188,000
5	4	662,000	179,000	483,000	26.7	98.8	83.0	166,000
6	3	2,316,000	1,243,000	1,073,000	52.5	97.8	95.4	772,000

Diet Development

Nutrient requirements for Florida pompano are poorly understood. Early investigations used diets formulated for other species, typically trout diets with 40% crude protein (CP), which resulted in good growth and survival, but poor feed efficiency (FE). Pompano are highly active, suggesting those early diets had insufficient digestible energy (DE) to support the high metabolic demand. Recently, it was determined that the optimal digestible protein (DP) and DP:DE ratio for juvenile pompano in saltwater (SW) are 360 g kg⁻¹ DP and between 23.8 and 25.1 mg DP kJ⁻¹ (Riche, 2009).

Apparent digestibility coefficients of feed ingredients exist for only a few fish species and are limited for Florida pompano. Compounding the problem is evidence that salinity affects nutrient digestibility (Dabrowski *et al.*, 1986). Recent evidence suggests transcripts of amino acid solute carriers are differentially expressed in the gastrointestinal tract in pompano held in saltwater and low salinity (see next

section), which may result in differences in amino acid (AA) availability in the two environments. Nutrient availability and established nutrient requirements are needed to implement least-cost feed formulation of balanced diets.

Digestibility and AA availability values for soybean meal (SBM), soy protein isolate (SPI), and corn gluten meal (Riche and Williams, 2010), as well as poultry by-product meal, meat and bone meal, and distillers dried grains (Williams, 2008) exist for pompano adapted to both SW and low salinity. Digestibility of CP and AA availability were high for the soy products but slightly lower for corn gluten meal. Digestible energy was inversely correlated to dietary carbohydrate, as is generally observed with carnivorous fish. The digestibility of CP (54-72%) and energy (64-76%) were lower and more variable for the by-product meals. In general, digestibility of the plant proteins appeared to be lower in SW than in low salinity water (Riche and Williams, 2010), but not for the by-product meals (Williams, 2008). To determine if verifiable differences in the

availability of nutrients exist between salinities we have designed a trial to evaluate seven additional ingredients in pompano held at 28 g l⁻¹ and 3 g l⁻¹. Sample analysis is ongoing. This will result in expanding the database of digestibility values to 17 ingredients for pompano in both saltwater and in water of low salinity.

Riche and Williams (2011) evaluated SBM and SPI as fishmeal (FM) replacements in diets fed to pompano reared at 3 g l⁻¹ salinity. Soybean meal could replace up to 80% and SPI 20% of FM protein without loss of growth or FE. Additional trials were conducted to evaluate nitrogen (N) utilization (digestibility, ammonia and urea excretion, and N accretion) and oxygen consumption in pompano fed five different poultry processing co-products as partial FM substitutes. No differences in growth, FE, or CP digestibility were detected relative to the FM control, and minor but significant differences were observed in ammonia and urea excretion. This suggests that poultry processing co-products are well utilized by pompano, even without indispensable the amino acid supplementation required by other species (Rawles *et al.*, 2006). However, establishing AA requirements for pompano will increase FE and decrease waste production.

Following evaluation of test diets utilizing three AA profiles (fish meal, pompano egg, and pompano

whole body) and two intact protein sources (corn gluten meal and casein-gelatin) we developed a suitable test diet for determining AA requirements utilizing casein-gelatin supplemented with purified AA to match the pompano whole body profile (Riche and Williams, 2007). Recently, we determined the lysine (Lys) requirement of pompano reared at 3 g l⁻¹ salinity using a traditional dose-response experiment. A broken saturated kinetics model was applied to the weight gain response and the Lys requirement was established as 22.5 g kg⁻¹ diet (20.0 g available Lys). The Lys requirement and the ratio of Lys to total indispensable AA in pompano whole body (Kaushik, 1998) were used to estimate the requirements for the remaining AA (Table 2). The estimated AA requirements can be used to formulate more efficient diets for pompano until the true requirements can be established.

In the future we will: 1) continue to develop nutrient digestibility values for traditional and novel feed ingredients for pompano; 2) establish the total sulfur AA and arginine requirements for pompano in low salinity water and seawater; 3) reduce reliance on FM and fish oil during pompano production; and 4) evaluate dietary strategies to improve growth and performance of marine fish reared in low salinity environments.

Table 2. Florida pompano whole body IAA¹ and estimated IAA requirements

Amino Acid	Whole Body Profile (%)	Estimated Requirement (g kg ⁻¹ diet)
Arginine	3.40	20.6
Histidine	1.15	7.0
Isoleucine	2.05	12.4
Leucine	3.52	21.3
Lysine	3.73	22.5
Methionine	1.69	10.2
Phenylalanine	1.90	11.5
Threonine	2.29	13.9
Tryptophan	0.22	1.3
Valine	2.39	14.5

¹ Mean based on analysis of 3 fish.

Physiological Adaptation of Marine Fish to Low Salinity

Rearing marine species in low salinity water has been established by the U.S. aquaculture industry as a priority research area for the development of sustainable commercial production of marine fish. Although we have successfully reared Florida pompano to market size at 5 g l⁻¹ salinity, we have observed that some populations of pompano exhibit low-grade chronic mortality when reared in low salinity environments. The cause of that mortality remains unknown. Interactions between salinity and fish health are complex and difficult to describe using conventional techniques. Genomic approaches using fish promise increased investigative power and have already provided insights into the mechanisms that underlie short-term and long-term environmental adaptations. Environmental genomics explores how the genome interacts with and integrates cues from the environment to produce both the effects of environmental stress and adaptive responses to stress. This approach can provide a description of genes involved in metabolic and osmoregulatory functions important for adaptation of pompano and other euryhaline marine species to low salinity conditions.

Advances in sequencing technologies have created the next generation of transcriptomics (Mardis, 2008). Similar to microarray, 454 sequencing can provide relative abundance of gene transcripts, but the method also provides species-specific sequences (Schirmer *et al.*, 2010). The method can provide insight into the molecular and physiological responses within cells and tissues to discover the complex physiological interactions between salinity and metabolism, growth, stress, and immune function.

Gill, kidney, intestine, and liver were individually collected from eight Florida pompano within three distinct groups; eight reared at 28 g l⁻¹ salinity (high salinity, HS), eight performing well (healthy) and eight performing poorly (moribund) following approximately eight months at 3 g l⁻¹ salinity (low salinity, LS). RNA from each tissue

sample was individually extracted using TRIsure™ (Bioline USA Inc, Randolph, MA). Total RNA was spectrophotometrically quantified and equal amounts of RNA from each of the eight fish within a group were pooled for each tissue. The resulting pooled samples were used to construct 12 native libraries: HS-gill, HS-liver, HS-kidney, HS-intestine, LS1-gill, LS1-liver, LS1-kidney, LS1-intestine, LS2-gill, LS2-liver, LS2-kidney, and LS2-intestine¹. Libraries were sequenced on a Roche GS-FLX (454) sequencer using Titanium chemistry (454 Life Sciences, a Roche Company, Branford, CT) by Purdue Genomics Core (Purdue University, West Lafayette, IN). Contigs (gene transcripts) were aligned from individual reads using Newbler 2.5 mapping and assembly software (Kumar and Blaxter, 2010). Contigs, isotigs and singletons were then annotated using BlastX and assigned gene ontology terms (GO-terms) using Blast2Go.

Gene transcripts, and their relative abundances, were compared between the HS healthy fish and LS healthy fish to identify potential effects of rearing pompano at LS (Table 3). Relative abundances of gene transcripts were also compared between the healthy pompano reared at LS with moribund pompano reared at the same salinity (Table 4) to discover potentially significant mechanisms that enable marine fish to successfully adapt to low salinities.

Gene ontology searches have to date focused on the abundance of solute carriers in the tissues known to play significant roles in osmoregulation: gill, kidney, and intestines. It appears that more solute carriers were up-regulated in kidneys of fish reared at 3 g l⁻¹ salinity while more were down-regulated in the intestines (Table 3). Carriers that transport the cationic amino acids Lys, arginine and ornithine were more abundant in the kidneys of healthy fish reared at LS, but less abundant in the moribund fish. Carriers that transport those same substrates appear to be down-regulated in the intestines of healthy fish reared at LS relative to both healthy fish reared at HS and moribund fish. Transcript abundance is currently being validated using qPCR. Future research will focus

¹ HS, high salinity (28 g l⁻¹); LS-1, low salinity healthy fish (3 g l⁻¹); LS-2, low salinity moribund fish (3 g l⁻¹).

Table 3. Solute carriers (SLC) altered in Florida pompano reared at low salinity

Solute Carriers	Tissue	Log (Δ TPM) ¹	Substrates
<u>Up-Regulated</u>			
SLC 12 member 6	Gill	3.95	potassium, chloride
SLC 12 member 9	Gill	3.95	potassium, chloride
SLC 7 member 6	Gill	3.95	cationic amino acids
SLC 7 member 7	Gill	3.95	cationic amino acids
SLC 7 member 3	Gill	4.26	cationic amino acids
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 24 member 3	Kidney	4.07	sodium, potassium, calcium
SLC 7 member 8 isoform 1	Kidney	4.07	cationic amino acids
SLC 7 member 7	Kidney	4.07	cationic amino acids
SLC 16 member 10	Kidney	4.07	aromatic amino acids
SLC 6 member 10	Kidney	4.37	neutral amino acids
SLC 24 member 2	Kidney	4.37	sodium, potassium, calcium
SLC 12 member 9	Intestine	4.68	potassium, chloride
SLC 6 member 19	Intestine	4.68	neutral amino acid transporter
<u>Down Regulated</u>			
SLC 24 member 3	Gill	4.32	sodium, potassium, calcium
SLC 7 member 9	Gill	4.32	cationic amino acids
SLC 7 member 2 isoform 1	Gill	4.02	cationic amino acids
SLC 12 member 2	Kidney	4.08	potassium, chloride
SLC 12 member 7	Kidney	4.08	potassium, chloride
SLC 24 member 6	Intestine	4.41	sodium, potassium, calcium
SLC 7 member 1	Intestine	4.41	cationic amino acids
SLC 7 member 2	Intestine	4.41	cationic amino acids
SLC 7 member 6	Intestine	4.41	cationic amino acids
SLC 12 member 6	Intestine	4.11	potassium, chloride
SLC 12 member 7	Intestine	4.11	potassium, chloride
SLC 3 member 1	Intestine	4.11	activator of dibasic and neutral amino acid transport
SLC 3 member 2 isoform 1	Intestine	4.11	activator of dibasic and neutral amino acid transport
SLC 7 member 4	Intestine	4.11	cationic amino acids
SLC 7 member 2 isoform 1	Intestine	4.11	cationic amino acids

¹Log₁₀ ((transcripts per million, from healthy fish reared at high salinity) - (transcripts per million, from healthy fish reared at low salinity)).

Table 4. Solute carriers (SLC) altered in moribund Florida pompano reared at low salinity relative to healthy fish reared at low salinity

Solute Carriers	Tissue	Log (Δ TPM) ¹	Substrates
<u>Up-Regulated</u>			
SLC 12 member 7	Gill	4.12	potassium, chloride
SLC 24 member 3	Gill	4.12	sodium, potassium, calcium
SLC 7 member 4	Gill	4.12	cationic amino acids
SLC 7 member 8	Gill	4.12	cationic amino acids
SLC 7 member 2 isoform 1	Gill	4.12	cationic amino acids
SLC 6 member 19	Gill	4.43	neutral amino acids
SLC 6 member 14	Kidney	4.14	amino acids
SLC 7 member 1	Kidney	4.14	cationic amino acids
SLC 36 member 1	Kidney	4.44	proton, amino acids
SLC 7 member 2	Kidney	4.62	cationic amino acids
SLC 12 member 6 isoform 2	Intestine	4.28	potassium, chloride
SLC 12 member 7	Intestine	4.28	potassium, chloride
SLC 24 member 6	Intestine	4.28	sodium, potassium, calcium
SLC 7 member 14	Intestine	4.28	cationic amino acids
SLC 7 member 3	Intestine	4.28	cationic amino acids
SLC 7 member 5	Intestine	4.28	cationic amino acids
SLC 7 member 6	Intestine	4.28	cationic amino acids
SLC 7 member 8	Intestine	4.28	cationic amino acids
<u>Down-Regulated</u>			
SLC 7 member 14	Gill	3.95	cationic amino acids
SLC 7 member 6	Gill	3.95	cationic amino acids
SLC 7 member 7	Gill	3.95	cationic amino acids
SLC 6 member 10	Kidney	4.37	neutral amino acids
SLC 24 member 2	Kidney	4.37	sodium, potassium, calcium
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 12 member 9	Kidney	4.07	potassium, chloride
SLC 12 member 5	Kidney	4.07	potassium, chloride
SLC 24 member 3	Kidney	4.07	sodium, potassium, calcium
SLC 7 member 5	Kidney	4.07	cationic amino acids
SLC 7 member 8 isoform 1	Kidney	4.07	cationic amino acids
SLC 7 member 7	Kidney	4.07	cationic amino acids
SLC 12 member 5	Intestine	4.68	potassium, chloride
SLC 12 member 9	Intestine	4.68	potassium, chloride
SLC 12 member 5	Intestine	4.68	potassium, chloride
SLC 6 member 19	Intestine	4.68	neutral amino acids

¹Log₁₀ ((transcripts per million, from healthy fish reared at high salinity)-(transcripts per million, from healthy fish reared at low salinity)).

on addressing the problem of low-grade chronic mortality at low salinity by utilizing nutritional, physiological, and environmental approaches.

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Land-Based Poly-Eco-Aquaculture of Abalone and Seaweed in a Small Scale Recirculating System Using a Recycled Freezer Container

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Abstract : To minimize environmental problems associated with aquaculture, we wanted to develop an abalone and seaweed polyculture approach in a small scale recirculating aquaculture system housed in an air-conditioned recycled freezer container. We conducted two experiments; each used two recirculating systems. Each system consisted of two biofilters and two abalone culture tanks. Each abalone culture tank contained three plastic baskets for abalone. In the first experiment one of the systems also incorporated a protein skimmer (PS) to evaluate its effects on water quality and abalone growth. In the second experiment, the same system was incorporated with both a PS and a seaweed culture tank (PSS) to evaluate their combined effects on water quality and abalone growth. The abalone stocking density was 20 individuals (average weight 5.3 ± 0.08 g and 8.7 ± 1.9 g in the first and second experiment) per basket. Pelleted artificial feed was supplied six days per week at 2.3% of abalone body weight per day. The pH, dissolved oxygen (DO), total inorganic nitrogen (TIN), total inorganic phosphorus (TIP), and bacterial abundance were monitored daily. The duration of first and second experiments were 87 and 70 days. DO concentration was significantly higher in the system with the PS. An opposite trend was observed in TIN concentration and bacterial abundance. PS had no effect on pH or TIP. PSS influenced water quality parameters and bacterial abundance similar to PS except TIP, which was greater in the system with PSS than without. Treatment effects on growth, feed consumption, and FCR were similar in both experiments. Abalone consumed less feed and had significantly higher FCR and lower growth rates in the control. However, feed consumption, FCR and growth rate of abalone were comparatively better in the PSS system than in the PS system. The PSS system was not only better for abalone growth, but also produced an additional crop in the form of seaweed. The system did not discharge waste. Therefore, future abalone culture systems can be focused on this model. However, more research is necessary before extrapolating results to an industrial level.

Key words : poly-eco-aquaculture, abalone, seaweed, recirculating system

Introduction

Abalone is one of the most expensive seafoods (Qi *et al.*, 2010). Because of high demand and price, wild abalone fisheries in many regions are declining as a result of over-exploitation (Alcantara and Noro,

2006; Taylor and Tsvetnenko, 2004). Decreased wild catches, combined with increasing demand for abalone have accelerated the development of their aquaculture (Coote *et al.*, 2006). Abalone aquaculture began in Japan over 60 years ago (Hone and Flaming, 1997). Since then, abalone aquaculture

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has been developing with the goal of maximizing production and minimizing cost. However, in most cases, aquaculture impacts the surrounding ecosystem through habitat destruction, waste discharge, and pathogen invasions (Barg, 1992; Wu, 1995; Naylor *et al.*, 2000). Therefore, awareness is growing in the society about the negative impacts on marine ecosystems, which puts increasing demand on environment friendly aquaculture.

In Japan, abalone is generally cultured in coastal floating net cages and in land-based flowthrough systems (Alcantara and Noro, 2006; Pereira and Rasse, 2007). In conjunction with floating net cages, farmers face many obstacles related to predation, poaching, weather conditions and offshore mariculture regulation. In addition, floating net cages discharge waste directly into the marine environment. Although land-based flowthrough systems reduce some obstacles related to predation, poaching and weather conditions (Leonard, 1993), they also discharge high nutrient-rich waste directly or indirectly into coastal waters (Hall *et al.*, 1992; Islam, 2005). Moreover, land-based flowthrough systems need a large area of land, which is often difficult to find in a country like Japan. Another critical problem for abalone culture in land-based flowthrough systems is maintaining optimum water temperature especially during the winter season (Nie *et al.*, 1996; Troell *et al.*, 2006). These problems reduce the economic success of land-based flowthrough systems.

To minimize environmental problems and the control of water temperature, a new approach to abalone aquaculture was proposed: a small scale recirculating aquaculture system housed in an air-conditioned recycled freezer container. Such a system should enable treatment of wastewater and allow complete environmental control. The system could confer ecological and economic advantages by reducing the amounts of water, energy and land use required. Comparatively little space and costs would be required compared to flowthrough systems. Importantly, systems could be established far from expensive coastal areas and use artificial seawater prepared from sea salt and freshwater.

Materials and Methods

System design: We conducted two experiments, each with two recirculating systems housed in a recycled freezer container ($4.3 \times 1.9 \times 1.9$ m) at the Kamoike Marine Production Laboratory, Faculty of Fisheries, Kagoshima University, Japan. Each recirculating system consisted of two biofilters (100 and 200 L) and two abalone culture tanks (200 L each). A schematic diagram of the systems is presented in Figure 1. One of the systems incorporated a PS to evaluate the effects of it on water quality and abalone growth (first experiment). The same system was incorporated with both a PS and seaweed culture tank to evaluate the effects of the combination on water quality and abalone growth (second experiment). Initially, 20 g of seaweed (*Ulva pertusa*) was stocked in the seaweed tank. Seaweed was partially harvested when its biomass exceeded 100 g.

The biofilter tanks were continuously aerated from the bottom through 25 mm PVC tubing with 1 mm perforations every 5 cm. Each abalone culture tank contained three plastic baskets (each $50 \times 34 \times 6$ cm) with a mesh size of 12 mm. Thus, each recirculating system had six plastic baskets. An air-conditioner (1.1 kwh) was used to maintain the desired water temperature ($19.2 \pm 0.8^\circ\text{C}$ for both experiments). Artificial sea salt was obtained from Marine-Tech Ltd., Japan and mixed with freshwater up to desired salinity (32.9 ± 0.2 ppt). Water circulation during both experiments was maintained at 43 L/minute. The first experiment was conducted for 87 days between March and August 2009 and the second experiment for 70 days between October and December 2009 with zero water exchange.

Abalone stocking and Management: Twenty hybrid abalone (*Haliotis discus hannai* $\times$ *H. sieboldii*) averaging 5.3 ± 0.08 g and average shell length 3.5 ± 0.8 cm (first experiment) and 8.7 ± 1.9 g and average shell length 4.0 ± 0.2 cm (second experiment) were stocked in each plastic basket. All abalone were collected from the Kosumo Ocean Ranching Co. Ltd., Japan. Abalone were fed pelleted feed containing 34% crude protein, 4% fat, 1.6% calcium, 1% phosphorous and 20% total mineral six days per week at 2.3% of body weight daily

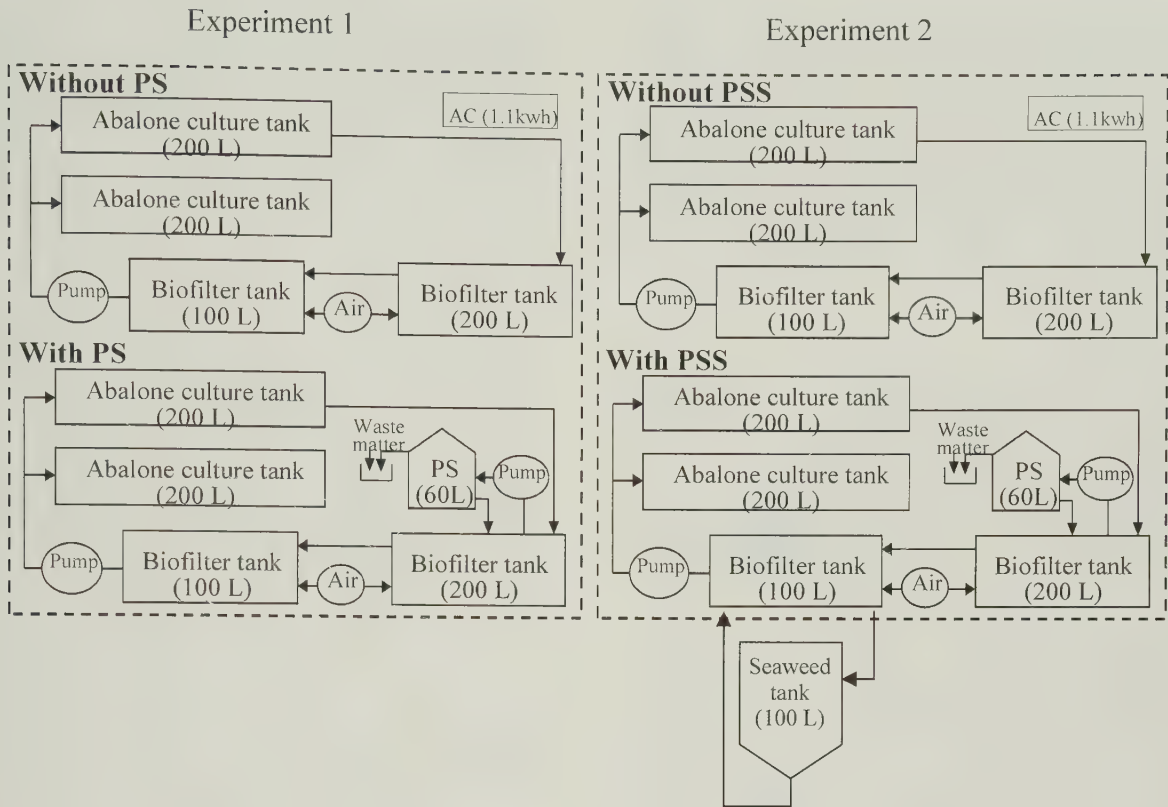


Fig. 1. Schematic diagram of recirculating systems used in experiment 1 and 2. Dotted line indicates container (4.3 × 1.9 × 1.9 m), PS indicates protein skimmer and PSS indicates protein skimmer plus seaweed.

starting the day after stocking until the day prior to harvesting. Feces and uneaten feed were collected next day of feeding.

Water Quality Analysis: Dissolved oxygen (DO), pH and all inorganic nitrogen and phosphorus species in the abalone culture baskets were monitored daily between 1000 and 1100 hours from the day before stocking until the day of harvest. Digital meters were used to measure DO and pH. Inorganic nitrogen and phosphorus species were quantified using a HACH DR/2000 spectrophotometer (HACH Co., Loveland, Colorado USA).

Total Bacterial Load Assessment: The bacterial loads were estimated following the same schedule used to determine water quality. Water samples were collected in sterile glass bottles from each basket for transport to the laboratory. Total count water testers (Millipore S.A.S. 67120 Molsheim, France) and an incubator were used to estimate total numbers of bacteria/ml of water.

Abalone Harvesting: All abalone were individually

measured (both length and weight) at the end of the experiment. Growth in length was calculated for each basket using the formula: *Growth in length* ($\mu\text{m}/\text{day}$) = $(L_f - L_i)/T$, where T is in days, L_f is the length at the end of the experiment and L_i is the length at the beginning of the experiment. Weight measurements were used to calculate specific growth rates (SGR, % body weight day^{-1}), using the formula of Day and Fleming (1992): $\text{SGR} = [\ln WT_f - \ln WT_i] \times 100/T$, where WT_f is the average final weight (g), WT_i is the average initial weight (g), and T is the duration of the experiment (days). Food conversion ratio (FCR) was calculated from feed consumption and growth in weight using formula: $\text{FCR} = \text{feed intake (g)}/\text{weight gain (g)}$.

Data Analysis: Data were analyzed using SAS version 8 (SAS Institute, Inc., Cary, North Carolina USA). A t-test was performed to compare main fixed treatment effects (first experiment: PS vs. no PS; second experiment: with PS and seaweed vs. without PS and seaweed) on different growth

parameters. A repeated measure ANOVA was used to examine temporal changes in water quality parameters and heterotrophic bacteria abundance for each treatment. The assumptions of normality and homogeneity of variance were tested. The data were transformed to satisfy normality if required. Differences were considered significant at $P \leq 0.05$.

Results

Effects on Water Quality Parameters and Bacterial Abundance: Table 1 shows the mean, maxima, minima and ANOVA results for pH, DO, total inorganic nitrogen (TIN), total inorganic phosphorous (TIP) and total bacterial abundance. First and second experiments showed similar treatment effects on water quality parameters and bacterial abundance except TIP. In the first experiment, PS significantly influenced DO, TIN and bacterial abundance, but had no impact on pH and TIP (Table 1). DO concentration was significantly higher in the system with PS than without. An opposite trend was observed in TIN concentration and bacterial abundance. PS increased DO concentration by 7% and decreased TIN concentration by 15% and bacterial abundance by 60%. In the second

experiment, PSS increased DO concentration by 21% and decreased TIN concentration by 15%, TIP concentration by 22 % and bacterial abundance by 49%. All water quality parameters and bacterial abundance also varied significantly over time in both experiments (Table 1). However, in the first experiment, there was no interaction effect of experimental period and PS on any water quality parameter except TIN concentration and bacterial abundance. In the second experiment, there was no interaction effect of experimental period and PSS on any water quality parameter or bacterial abundance.

Effects on Growth, Feed Consumption, FCR and Survival of Abalone: Treatment effects on growth in length, SGR, feed consumption and FCR were similar in both experiments when compared with the control in each system (Figure 2). Abalone consumed less feed and had significantly higher FCR and lower growth rates (greater shell growth and higher specific growth rate) in the control system than in the other system. However, abalone feed consumption, FCR and growth rate were comparatively better in the PS plus seaweed system (second experiment) than in the PS system (first experiment). Treatment effect on abalone survival was not significant ($P>0.05$) in either experiment.

Table 1. Water quality parameters in abalone tanks of experiments 1 and 2 based on one-way repeated measure ANOVA. Ranges are presented in parentheses.

Variable	Experiment 1 (Effects of PS)					Experiment 2 (Combined effects of PSS)				
	Significance (<i>P</i> value)			Treatment means $\pm$ 95% confidence intervals		Significance (<i>P</i> value)			Treatment means $\pm$ 95% confidence intervals	
	PS	Time	PS $\times$ Time	Without PS	With PS	PSS	Time	PSS $\times$ Time	Without PSS	With PSS
pH	NS	*	NS	8.14 $\pm$ 0.03 (7.66–8.35)	8.16 $\pm$ 0.03 (7.71–8.34)	NS	**	NS	7.84 $\pm$ 0.02 (6.20–7.91)	7.94 $\pm$ 0.02 (5.90–7.97)
DO (mg/L)	**	**	NS	6.6 $\pm$ 0.04 (6.0–7.8)	7.1 $\pm$ 0.04 (6.2–8.1)	**	**	NS	6.03 $\pm$ 0.10 (4.69–8.24)	7.30 $\pm$ 0.05 (5.71–8.54)
TIN (mg/L)	*	**	**	4.38 $\pm$ 0.23 (2.11–4.93)	3.73 $\pm$ 0.20 (1.61–4.34)	**	**	NS	19.13 $\pm$ 0.90 (4.30–34.22)	16.21 $\pm$ 0.84 (2.41–20.12)
TIP (mg/L)	NS	*	NS	0.22 $\pm$ 0.01 (0.10–0.41)	0.20 $\pm$ 0.01 (0.08–0.37)	**	*	NS	3.34 $\pm$ 0.46 (0.39–5.40)	2.59 $\pm$ 0.29 (0.29–5.85)
Bacteria Abundance ($\times 10^5$ CFU/mL)	*	*	*	2.64 $\pm$ 0.62 (0.001–6.60)	1.03 $\pm$ 0.28 (0.001–4.30)	*	**	NS	6.20 $\pm$ 1.18 (0.001–12.90)	3.18 $\pm$ 0.97 (0.001–7.20)

* $P \leq 0.05$; ** $P < 0.01$; NS, not significant; PS, protein skimmer; PSS, protein skimmer plus seaweed; Time, effects of sampling period; TIN, total inorganic nitrogen; TIP, total inorganic phosphorous.

Discussion

Heating is a common method of maintaining water temperature in recirculating systems (Nie *et al.*, 1996; Masser *et al.*, 1999; Martins *et al.*, 2009), but is typically very costly and reduces the economic

viability of the culture system. In the present study, we used an insulated container and an air conditioner (AC), which successfully maintained optimum water temperature (range 18–20°C; mean: $19.2 \pm 0.8^\circ\text{C}$) in both experiments without significant differences between treatments. The study suggests

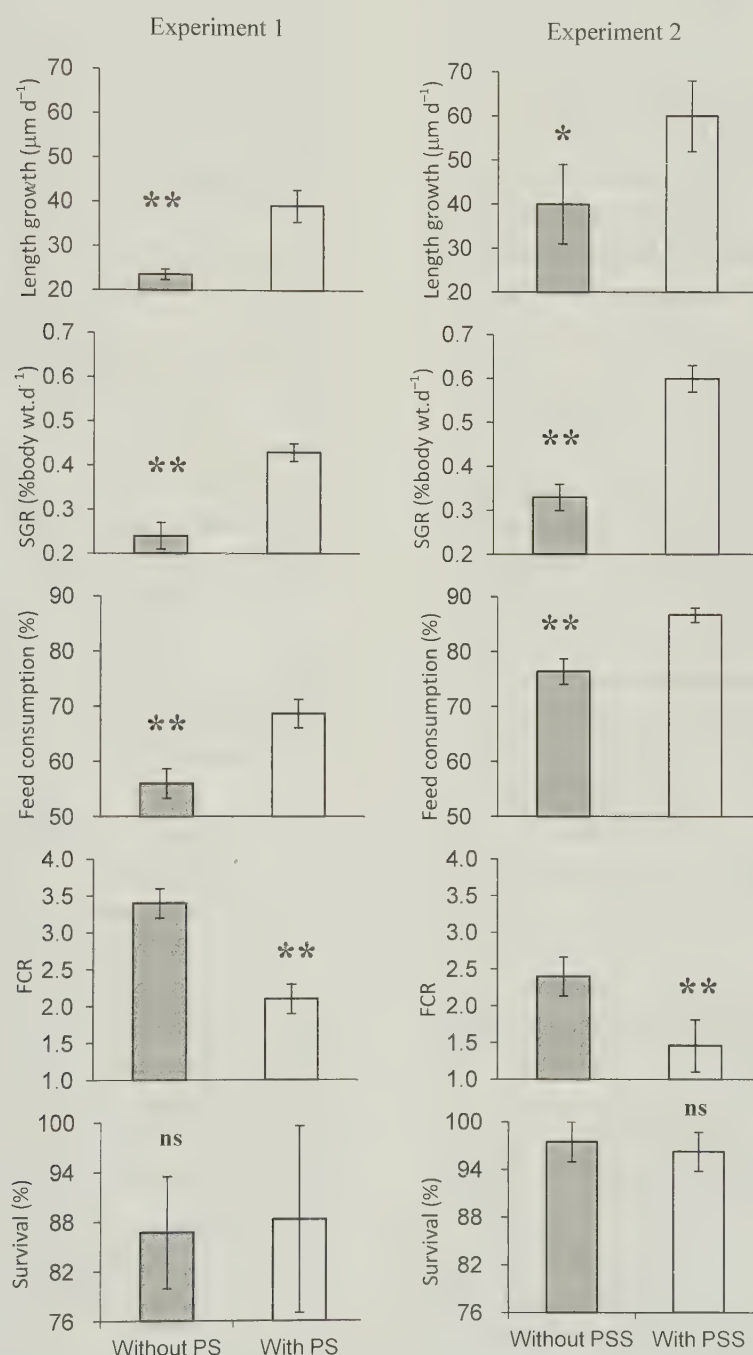


Fig. 2. Growth, feed consumption, FCR and survival in experiments 1 and 2 based on t-test. Data are mean $\pm$ 95% confidence intervals. Asterisk (* $P \leq 0.05$; ** $P < 0.01$) represents significant treatment difference and ns represents no significant treatment difference.

that an insulated recycled container and an air conditioner can be used to maintain suitable water temperature in recirculating systems for abalone aquaculture.

In the present study, protein skimming reduced waste matter and subsequently aerobic bacterial decomposition, resulting in lower TIN concentrations in the system with a PS alone or a PS along with seaweed (Sugawara and Kadowaki, 2006; Rahman *et al.*, 2008). Protein skimming reduced organic waste and bacterial abundance, resulting in less decomposition, lower TIN concentrations and higher DO concentrations in system with PS or PS and seaweed compared with the control systems. The lower decomposition and TIN and higher DO can also explain why the abundance of bacteria in the water was lower in system with PS or PS and seaweed than in systems with neither. The influence of PS and seaweed on DO concentration was more pronounced than the influence of PS alone on DO concentration. The reason might have been due to the addition of the seaweed culture tank with a PS. Seaweed produces oxygen, which increased dissolved oxygen concentration in the system with seaweed (Kadowaki, 2004).

DO and TIN concentrations may have been the most significant environmental factors influencing abalone feed consumption, FCR and growth in the present study. All of these growth parameters were higher in the two systems that had a PS. There are no previous studies comparing the effects of a PS on abalone food consumption and growth in recirculating systems, but Sano and Maniwa (1962) reported decreased food consumption and growth of *Haliotis discus hannai* with increasing TIN concentration. Similar effects of TIN on feed consumption and growth were also observed in greenlip abalone (*H. laevisgata*) by Harris *et al.* (1998). Previous studies have suggested frequent water exchange as a method for managing TIN concentrations in traditional land-based abalone culture (Capinpin *et al.*, 1999; Evans and Langdon, 2000; Badillo *et al.*, 2007). However, frequent water exchanges increase operating costs and the present study suggests protein skimmers may present a viable alternative to water changes for maintaining water quality in recirculation systems for abalone

culture.

Addition of a PS skimmer and seaweed culture tank resulted in comparatively better abalone growth than the abalone growth in a recirculating system with only a PS. This result concurs with Kadowaki (2004), who reported better seaweed and fish growth near a seaweed culture area than away from the seaweed culture area. According to him, seaweed utilize inorganic nitrogen and phosphorus, inhibit pathogenic bacteria and produce oxygen. All of these influence abalone growth. Abalone growth is extremely slow and often varies with size and age (Steinarsson and Imsland, 2003; Naidoo *et al.*, 2006). Although several previous studies have examined the growth of *H. discus hannai* (e.g., Neori *et al.*, 2000; Wu *et al.*, 2009; Qi *et al.*, 2010), none have focused on growth of the hybrid used in this study. However, we observed comparatively higher growth (SGR = 0.60 %/day) in the system with a PS and seaweed if we compare that SGR with those from other studies. Neori *et al.* (2000) reported a SGR for 44.2 mm *H. discus hannai* fed on *Ulva* sp. at 0.34%/ day. Wu *et al.* (2009) observed SGR 0.24-0.33%/day for 23.4-34.9 mm *H. discus hannai* fed *Gracilaria lemaneiformis* and *Laminaria japonica*. Qi *et al.* (2010) observed a very low SGR range of 0.11-0.12%/day in 7.6 cm *H. discus hannai* fed various seaweed diets.

Conclusion

The PS with seaweed system not only improved abalone growth, but also produced an additional crop in the form of seaweed. The system also did not discharge waste. Therefore, future abalone culture systems can be focused on the described system. However, more research (optimization of abalone density and optimization of seaweed biomass) is necessary before extrapolating the results to an industrial level.

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Pond-to-Plate Analysis of the U.S. Farm-Raised Catfish Industry

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Abstract : U.S. domestic production and sales of catfish have declined from 300.3 million kg of processed catfish in 2003 to 213.6 million kg in 2010, while during the same period imports of tilapia, basa, tra, and channel catfish have increased. Several factors contributed to the decline, including lower import fish prices, increasing domestic feed/fuel prices, inefficiencies in U.S. catfish production, and inconsistencies in domestically produced products. A proactive focus on industry improvement at all levels is needed. A Pond-to-Plate project was initiated in 2009, with the goals of improving the competitiveness of the US farm-raised catfish industry and evolving it into a modern livestock industry. Auburn University's Fisheries Department has brought in LEAN trainers from the College of Business to assist in conducting Pond-to-Plate meetings in west Alabama. This project uses the LEAN manufacturing and continuous improvement concept and has been introduced at Pond-to-Plate meetings held in west Alabama. Each meeting includes participant representatives of the value chain; i.e., catfish producers, harvesters, transporters, processors, distributors and consumers. The LEAN enterprise produces more with existing resources by eliminating non-value-added activities. Manufacturers are facing increased worldwide competition and the stakes are high. The winners in this competition work to eliminate overproduction caused by traditional scheduling systems and to only make what customers want when they want it. Lean establishes a systematic approach to eliminate these wastes and create a flow throughout the whole company. It also helps companies develop and implement a long-term plan to streamline their operations for success. Training uses a hands-on approach involving a mixture of the company's management and staff members. This approach was modified to address the fact that the U.S. catfish industry is not one company but is comprised of independent producers with few formal ties to processing plants. Catfish Pond-to-Plate meetings have used LEAN principles to address key issues of industry efficiency at each level of the value chain, increasing demand for catfish products, lack of product/value informational flow, final customer/consumer needs/desires, and non- or misaligned objectives, product quality needs, and incentives/rewards among value chain members to produce consistently high quality products. Meetings have resulted in articulated vision statements addressing the key issues identified and have focused on activities to reach the stated goals of increasing per capita consumption of U.S. farm-raised catfish.

Key words : aquaculture, catfish, LEAN manufacturing, yellow fillets, technology

Introduction

The U.S. farm-raised catfish industry has been declining since 2003. In 2003 there were 300.3 million

kg of catfish processed in the U.S. and in 2010 there were 213.6 million kg processed, a decline of 86.2 million kg or 29%. Likewise, there was a similar decrease in pond production area, from 75,757 ha

in 2003 to 46,458 ha in 2010, a decline of 29,299 or 39%. It is generally accepted that the decline was primarily due to the growth of U.S. consumption of other fish species, which are primarily imported (the U.S. imports 83% of all the fish and seafood consumed). U.S. farm-raised frozen catfish fillet sales have been losing ground to imported frozen catfish fillet sales, but imports of frozen tilapia fillets have also taken market share from the U.S. catfish industry. Also, input costs for feed and fuel have made it more expensive to produce catfish domestically and has added to the decline in domestic sales of frozen product. Another factor is the catfish product image. Many Americans have a negative image of catfish, coming from childhood experiences fishing for bottom dwelling catfish that had a muddy flavor. This is not the case with aquacultured catfish that feed on nutritionally complete feeds that float, but occasionally some fillets with musty or earthy flavors get into the market. Such flavor inconsistencies negatively affect consumer opinions toward catfish, creating an image that is difficult to overcome; thus, it is necessary to engage in standard operating practices and best management practices that provide a high quality product each and every time it is purchased. Processors need a consistent, high quality product to entice consumers to continue to buy their product as consumers have many protein choices from which to choose.

Pond to Plate Project

The Pond-to-Plate approach uses multiple funding sources to work on all objectives simultaneously as they are interconnected. The project is advised by a seven-member task force and a 25-member core industry group of producers, harvesters, haulers, processors, wholesalers, retailers, researchers, and extension workers in a cross-disciplinary research and extension effort. The project has utilized the services of LEAN manufacturing experts to facilitate Pond-to-Plate meetings. The choice of LEAN manufacturing and continuous improvement techniques was chosen as one of many approaches used to reach our project goals and is the focus of this report. LEAN manufacturing is defined as "...

a systematic approach to identifying and eliminating waste, i.e., non-value-added activities, through continuous improvement by flowing the product at the pull of the customer in pursuit of perfection." By the end of 2010, six Pond-to-Plate meetings had been held with catfish producers, harvesters, transporters, processors, and buyers participating.

The six workshops resulted in a characterization of the industry value chain, literally from pond production to consumption by final consumers (production, processing, distribution, retail). In this process, wastes (inefficiencies) along the value chain as well as identification of strengths, weaknesses, opportunities and threats to the industry were identified. These exercises allowed participants to work together to see how standards and management practices must be developed with the next levels product needs in mind to achieve the ultimate goal of providing what the consumer desires. To reach this goal the U.S. catfish industry must be transformed into a modern livestock industry taking advantage of U.S. strengths in technology and innovation. Ultimately, growing the U.S. farm-raised catfish industry in size and profitability will require improvements in production/processing efficiency to reduce costs, identifying and eliminating wastes, improving product quality and consistency to compete with substitute (imported) products, and promotion at each value stream level. A market pull situation where consumers demand products with recognized attributes is desired as opposed to the current market push approach that "pushes" processed products into the market place whether they are in high demand or not. There are many seafood and other protein choices in the marketplace and farm-raised catfish is competing against each and can be measured through consumer's vote which is done through their purchase decisions.

From the Pond-to-Plate meetings, five teams were created to address critical issues along the market/value chain, including: 1) product quality, consistency, and production systems; 2) consumer awareness; 3) packaging and product development; 4) branding and imaging; and 5) byproduct development and utilization. Meeting attendees began to see that they are all participants in the

entire value chain, whether they be producers, harvesters, transporters, processors, distributors, or retailers and their actions in developing best management practices (BMP) and standard operating procedures (SOP) at critical control points are important to the overall health of the industry.

The teams have become active in identifying priority issues. For instance, the yellow fillet problem has quickly become a top priority of the product quality, consistency, and production system (Team 1) and survey efforts are underway to connect product quality with production management strategies. Finding a solution to the yellow pigment problem in the short term will provide a model for expediently addressing other problem areas that challenge the industry. In this manner other problem areas will be addressed and BMPs and SOPs will be developed through industry input to achieve efficiencies in production and consistent quality products leading to a competitive industry that can compete with world seafood imports. In the following sections a brief summary of some of the research efforts being conducted at each level of the industry market value chain are described.

Production Efficiency

Yield verification trials are being conducted on commercial farm ponds and in small-scale experimental ponds at the E.W. Shell Fisheries Station (Auburn University research ponds). Trials are being conducted according to the protocol developed using currently best-known production practices and compared to current farm practices. At present, there are three west Alabama catfish farms participating in this program. The goal is to compare and evaluate existing farm management strategies to BMPs developed by researchers and extension personnel. The difference between this approach and prior yield verification studies is this research is measuring product quality data such as yellowness in the fillet as well as production efficiency. Both areas must be addressed to reach the final goal of meeting or exceeding customer expectations.

A yield verification protocol was established for use in farmer participant ponds and Auburn

University experimental ponds in early 2010. Data were collected on initial stocking, feed input, tractor/aeration/feeding labor hours, and weekly water quality data from each pond. Additionally, data on chemicals applied, labor used, other operating inputs, and pounds harvested/sold will be collected. Note that catfish stocked as 15.24 cm fingerlings can take 18-24 months to reach 1.8 kg harvest size, so no results on production data will be reported. A data spreadsheet has been developed to enter data that will calculate ongoing production expenses and receipts to provide accumulated costs that will be useful for catfish farm managers who take the time to enter data and use the information to assist them in making management decisions. The overall goal is to biologically and economically compare and demonstrate the efficiency of BMP management strategies with previous management protocols and to involve producers in the process.

New Technology

A second area of industry modernization involves the introduction of new technologies to the pond production process through on-farm demonstrations. Technologies introduced during the project include a bar grader for use with hybrid catfish. The technology can have significant impacts on the industry as it allows for ease in sorting hybrid channel catfish (a faster growing, disease resistant fish) compared to the traditional harvest seines and live cars where hybrid fish get hung up in the net, requiring many additional hours of work. The hybrid catfish is relatively new to the industry though its advantages have been known for 30 years. Only in the last 5-10 years have enough hybrids been produced annually that producers have been able to purchase them on a regular basis. Yet, as of 2010 only 35 million hybrid fingerlings were produced compared to the one billion fingerlings required by the industry annually. The bar grader net is one of the yield verification requirements for harvesting fish on participating producer ponds. The net allows smaller fish to escape through the bars and remain in the pond until the right harvest size is reached, while taking out larger fish that need to be harvested for the sake of efficiency. In addition,

the new bar grader works well with channel catfish. The bar grader and accompanying seine net have been made available on a check-out basis to all catfish producers in west Alabama. Producers who have used the grader/net have begun to use it on a routine basis. This is progress, as word of mouth on the effectiveness of the gear is being passed on to other producers.

The second new technology being introduced to west Alabama aquaculture producers is the in-pond raceway system (IPRS) concept (Brown *et al.* 2010). The system can use existing pond infrastructure and has the potential to provide producers with inventory control, a critical factor missing in the multiple-batch pond production system. Other benefits, such as improved feed conversion ratio and higher production levels occur from tighter control over inventory and rapid, efficient control of diseases through providing medicated feed only to fish that show disease signs. Fish can also be segregated by size, allowing higher protein feeds to be provided to smaller fish without competition from larger fish, as occurs in traditional pond aquaculture. Issues from multiple batch production systems can be ameliorated by IPRS: lower mortalities from bird depredation can be achieved; starvation or malnutrition of smaller fish can be avoided; losses from other predators can be avoided; diseases can be effectively treated in a much smaller area; and reduced harvesting costs can be achieved. Overall, these systems can be more efficient to operate resulting in a lower cost of production and these systems are more flexible to operate than pond systems. Raceways can be completely harvested (100%) or fish can be harvested, graded, and moved to an adjoining raceway. Both are pathways to resetting the U.S. catfish industry onto a more profitable, competitive, and sustainable future and moving the pond aquaculture industry in the U.S. into a modern livestock industry.

Yellow Fillet Coloration

An emerging research area this project has fostered is investigation into the root causes of yellow fillet coloration. It appears that bioaccumulation of pigments coming from feed and

consumed forage fish that have ingested pigmented algae are accumulated in the catfish flesh resulting in yellow coloration (Li *et al.* 2007, 2009; Lovell 1984). Inconsistent coloration of catfish fillets, either yellow or red (from stress), when whitish coloration is desired, was identified as a major detrimental issue by processors as it restricted them from maintaining or expanding fresh fillet sales to grocery store outlets. Critical control point effectiveness affecting product quality and consistency has focused on yellow fillet reduction at the production, harvesting, transporting, and processing phases.

Research into yellow and red fillet pigmentation causation will allow the U.S. to develop management strategies to deal with this issue at the pond level. Collection of yellow fillet information from processing plants and producers began in early 2010 and is continuing to obtain a full year of data for analysis since seasonality affects the yellow coloration. Correlation of catfish production variables and processor identified yellow fillet percentages by pond harvest is the measure being used to identify significant production factors affecting fillet coloration. Producer surveys were initiated for each batch of fish delivered to two processing plants in west Alabama and are matched up to processor records on percentage of yellow fillet occurrence. The producer and processor data are entered into a database for statistical analysis. Detection of significant variables will be used to develop hypotheses and research plans to reduce and/or eliminate fillet color quality issues.

A second approach to the yellow fillet coloration issue has investigated bio-film development to stop or retard additional yellow fillet color intensification of the fresh (ice/refrigerated) product during the period from completion of processing to final purchase by consumers in the grocery store outlet. Ongoing efforts to chemically diminish and retard color development with an FDA approved wash solution are continuing. Preliminary results show promise though yellow color has not diminished enough to satisfy grocery store patrons. It should be noted that not all grocery store buyers and their customers see the yellow coloration as a problem. However, consumers do ask whether fillets that are white, yellow, and red in the same seafood display

case are safe; i.e., are yellow fillets rancid? They are not, but perception is the basis for purchasing decisions and thus the need for consistent products.

Yellow coloration is not as great a concern for restaurant and institutional buyers as they further process or cook the catfish product and no yellow coloration remains. Thus, methods to detect fillet coloration, measure the discoloration, and sorting the range of colored fillets has been identified as a constraint to efficiently selling the right product to the right buyers.

Quantifying the yellow coloration level to a numerical scale is being developed and used to grade filet color using colorimetric equipment (Leggett 2008, Schmehling 2008, Leon *et al.* 2006). Installation of color measurement equipment into the catfish processing line to select fish for acceptable colors for different markets would be beneficial (Misimi *et al.* 2007). However, real issues of expense and what to do if one portion of the sorted fillets cannot be easily sold need to be addressed.

Pond-to-Plate follow-up meetings with processor employees involved in daily examination of catfish products for quality assurance (aroma and color) occurred and the digital color index developed in this project resulted in classification of a fillet color chart for testing with processors. The chart has a range of fillet color photos sorted into three categories or degree of coloration. Any individual fillet may have several colors present making classification difficult and variable due to the different requirements by buyers. The categorization of fillet colors is quantified using a numerical scale.

Color rating experiments in which 100 catfish fillets were to be sorted by color and put into one of the three color categories (gold/dark yellow, some light yellow, and white) was conducted. Each fillet was numbered and each participant wrote down into which category each fillet should be placed. After compiling the rating data there was only 69% agreement. This indicates that the ideal fillet color is not standardized. Training of processor employees in color rating is needed, and variation in color within a single fillet is high. There is great interest in future efforts to investigate colorimetric equipment being used on the processing lines — as is currently being done in the salmon industry — to select fish with

acceptable colors for different markets.

Live Fish Quality and Rapid Improvement Event

Another area of concern is ensuring live catfish product quality as fish are harvested, held for grading, and transported to the processing plant. When fish are stressed during this process, red coloration can be imparted to the fish flesh. The red filet issue is being investigated by fish pathologists with the objective of determining its root cause, quantifying the red coloration for grading purposes, and looking at pre-harvest, harvest, and in-pond holding stresses for strategies to avoid the occurrence of the problem. To address this issue the project implemented a rapid improvement event (RIE) over a three-day period at one processing plant with the goal of reducing dead-on-arrival fish, punctures, and necrotic tissue through improved handling. The RIE team of 8-10 employees identified factors that contributed to the reduced quality of the fillet during the harvesting/transport phase and developed plans to reduce product defects.

This event was led by LEAN manufacturing experts and facilitated root cause analysis by team members to identify and develop methods to improve practices resulting in an increased percentage of higher quality fish arriving at the plant. The harvest/holding/transport/receiving process can produce stress in the fish and result in red fillets once processed, which is another characteristic of a degraded product. Stressed fish can produce a pink/red fillet coloration and injury can result in red splotches. In addition, dead fish can have necrotic tissue redness. The goal of the RIE was to reduce those occurrences and the number of such cases arriving at the processing plant. The process was important because it was the team of participants that found ways to improve the product. Awareness by employees that they can impact and improve product quality is empowering, especially when the knowledge is linked to the success of the business and continued operation of the plant.

Processed Fish Defects and Rapid Improvement Event

Consistency of high quality product must be ensured during the processing of catfish. Another RIE example involved a three-day RIE at a west Alabama processing plant focused on reducing defects at a limited section of the catfish processing line. Defects can be either color variation, remaining fins, holes, incorrect cuts, or low yields. The event involved plant personnel from line workers to the plant manager and took advantage of the cumulative knowledge of the participants. Root cause analysis was used to draw out information about the problem areas. Practices to address the issues and develop a protocol for doing so were discussed. The result was a program of monitoring and sampling that reduced miscuts and improved yield through incentives. Before the RIE event, processing lines were monetarily awarded weekly bonuses based on the quantity of fish processed. After the RIE event the processing line with the most processed fish *and* fewest defects was awarded with the incentive bonus. This small change resulting from the RIE significantly increased the quality of the product coming from the plant as line workers were encouraging fellow workers to get it right so their bonus would not be endangered.

Catfish Traits Desired by Wholesaler/Middlemen/Retailer

The December 2010 Pond-to-Plate meeting included catfish producers, harvesters, transporters, processors, and buyers. The results of a market value chain survey, including results on what catfish attributes retail buyer's value, and potential profitability through delivery of desired products through "pull" marketing rather than "push" marketing were discussed. Preparation for the meeting involved surveying wholesalers and retailers of catfish products to determine what they and their customers wanted in terms of size, coloration, and so forth. Interesting additional comments from the surveys indicated that more in-store promotion and more contact with producers and processors were desired. Additionally, it was found that the

catfish product being put on the market was not all bad; in fact, it was found that there was tremendous U.S. product loyalty and buyers liked the current products, though that varied by outlet type. In the end, Pond-to-Plate participants better understood why their production methods matter, why their attention to quality mattered, and that there is the possibility of increased producer and processor sales resulting from their individual farm and processor-level actions. Again, they saw that improvements can be achieved through BMPs and SOPs at each level and at critical control points along the value chain.

Summary and Conclusions

Pond-to-Plate meetings and spin-off events such as the RIEs and other processor-producer events have been very important in identifying, researching, and developing improvements in the U.S. farm-raised catfish industry, including product quality and consistency, improving efficiency at each value step, and improving the understanding among industry participants that one link in the value chain does affect the next level and vice versa. Meetings have brought industry participants together to find common ground and common goals. All are seeing the need for BMPs and SOP's for efficiency, quality control, competitiveness, and profitability.

This is a long-term effort and initial activities and successes are building momentum and empowering producers, harvesters, transporters, and processors with the understanding that standardization and BMPs are key to consistent products and industry competitiveness and growth. Furthermore, the need to produce a product with the attributes that consumers demand is crucial for the industry to make strides toward a modern livestock industry. The support from several funding sources for this initiative has been pivotal to bringing disjointed biological, economic, and marketing studies into a meaningful Pond-to-Plate effort. Under the LEAN manufacturing and continuous improvement philosophy an industry will never reach perfection, but instead a process of chipping away at specific problems across the market channel span will continuously pay off as improvements in efficiency,

quality consistency, and pull marketing occur.

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Case Studies in Flatfish Stock Enhancement: A Multi-Year Collaborative Effort to Evaluate the Impact of Acclimation Cage Conditioning for Japanese Flounder, *Paralichthys olivaceus*, in Wakasa Bay, Japan

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Abstract : Japan is the most active country in the world with respect to flatfish stock enhancement, both in the range of species and number of fish released. In Japan, Japanese flounder or hirame, *Paralichthys olivaceus*, is the primary species represented in the annual flatfish catch; thus, hirame has been a paramount choice for both aquaculture and stock enhancement for decades and is, in fact, the most important stocked marine finfish in Japan. A total of approximately 25 million Japanese flounder are released yearly from federal, prefectural, and local hatcheries throughout the country. Conditioning flatfish to the natural environment before release may increase successful recruitment to the fishery, as fish trained for “wild” conditions may transition more easily and successfully upon release. Since 2008, Obama Station, National Center for Stock Enhancement, has conducted pre-release experimental acclimation cage conditioning for Japanese flounder (N = 10,000-80,000) in both the Takahama and Obama portions of Wakasa Bay, Japan. Fish were reared via the “Hottoke shi-iku” method, a simplified rearing process that boosts cultivation efficiency of fingerlings by reducing rearing time and manpower. Recaptured fish were acquired through a cooperative effort between researchers and local fishermen (both commercial and recreational). To date, more conditioned fish have been recaptured via fishermen’s catch than non-conditioned fish. Initial observations suggest that non-feeding individuals captured near the release sites may be weaker and more likely to be caught by small boat beam trawl (towing speed 1-1.5 knots) than actively feeding, translocating fish. Thus, higher speed shrimp trawlers deeper in the bay (towing speed 3-3.5 knots) and set/fyke nets may be better, non-biased indicators of fitness and intermediate stocking success.

Key words : flatfish, hirame, *Paralichthys olivaceus*, Hottoke shi-iku, stock enhancement

Introduction

Flatfishes (flounder, halibut, sole) are among the most desirable and highly priced fishes sold for human consumption (Howell & Yamashita, 2005). Although flatfishes have supported valuable fisheries throughout the world for centuries, catches of many species have steadily declined. Many female marine fish species are capable of releasing hundreds of

thousands of eggs annually but because of the vulnerability of the small, early life-history stages, there is high natural mortality, and few survive to maturity. Spawning and rearing flatfish in captivity and releasing the young (i.e. stock enhancement) may help augment natural populations.

Over the past 10 years, researchers in the U.S. have conducted small-scale, experimental releases of winter flounder, *Pseudopleuronectes americanus*

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(Fairchild, 2002; Potier, 2007), southern flounder, *Paralichthys lethostigma* (Tompkins, 2010; Sikes 2011), and summer flounder, *Paralichthys dentatus* (Kellison *et al.*, 2003) totaling approximately 46,000 fish. Japan, on the other hand, has been releasing flatfish as a fisheries management strategy for over 30 years. Releases of Japanese flounder (*Paralichthys olivaceus*), marbled flounder (*Pseudopleuronectes yokohamae*), barfin flounder (*Verasper moseri*), spotted halibut (*Verasper variegatus*), and at least four other species total over half a billion (Howell and Yamashita, 2005; Yamashita and Aritaki, 2010). Japanese flounder, or hirame, is the primary species represented in the annual flatfish catch; thus hirame has been a paramount choice for both aquaculture and stock enhancement for decades and is, in fact, the most important stocked marine finfish in Japan. A total of approximately 25 million Japanese flounder are released annually from federal, prefectural (state), local, and private hatcheries throughout the country (Tomiyama *et al.*, 2008).

To gain perspective on the feasibility of stocking flatfish as well as establishing effective strategies for large-scale releases, M. Walsh initiated collaboration with Y. Yamashita of Kyoto University's Maizuru Fisheries Research Station and Obama Station, National Center for Stock Enhancement, Fisheries Research Agency, in 2009 as part of her doctoral program at the University of New Hampshire. Our objective here is to present details of how and why we established this collaboration, as well as to share preliminary observations regarding cage conditioning for flounder stock enhancement.

Funding a US-Japan Collaboration

A number of funding opportunities (both US and Japan-based) exist for graduate students to engage in collaborations (Table 1). UJNR hosted a student exchange for aquaculture in 1998, however that funding opportunity has since been discontinued. The East Asia and Pacific Summer Institute (EAPSI), sponsored jointly by the National Science Foundation (NSF) and the Japan Society for the Promotion of Science (JSPS), offers an approximately three-month duration fellowship for U.S. graduate students to conduct summer research in Japan (as well as in other Asian-Pacific countries). Fulbright

offers highly competitive international scholarships lasting approximately 9-18 months (depending on host country). The Japanese Government offers longer-term (1-3 year) Monbukagakusho Scholarships for both undergraduate and graduate students, and the Japan Student Services Organization (JASSO) provides short-term opportunities (3 months-1 year) for foreign students to study or conduct research in Japan. NSF, JSPS, and Fulbright also provide opportunities for postdoctoral and professional level researchers to work in Japan, as well as for Japanese researchers and students to work in the U.S. The current collaboration with Obama Station was predominantly funded and initiated via EAPSI and Fulbright scholarships.

Acclimation Cage Conditioning for Flatfish Stock Enhancement

Hatchery reared flatfish exhibit irregular swimming, feeding, and cryptic (burial and color change) behavioral patterns compared with wild conspecifics, and these behavioral "deficits" are assumed to translate to increased predation risk once fish are released into nature (Furuta, 1996; Kellison *et al.*, 2000). Conditioning flatfish to natural stimuli before release can increase survival, and thus successful recruitment to the fishery, as fish trained for "wild" conditions may transition more easily and successfully upon release. (Kellison *et al.*, 2000; Sparrevohn and Støttrup, 2007). Predator-free acclimation cages may help flatfish adjust to the wild environment, establish burial skills, begin pigment change, recover from transport stress (Fairchild *et al.*, 2008a), and experience natural (live) food sources before full release into the wild. Obama Station has been examining the effects of acclimation cage conditioning for Japanese flounder since 2008 in order to establish whether the practice increases flatfish stocking success.

Methods

In brief, the main protocols were to raise flounder by the "Hottoke shi-iku" method — a low labor, high efficiency rearing technique (Takahashi, 1998), until they reached approximately 10-12 cm in late June/early July. Then, approximately half of the

Table 1. Graduate student funding opportunities for US-JPN collaboration

AWARD	SPONSORING AGENCY	DURATION	WEBSITE
East Asia and Pacific Summer Institutes (EAPSI)	National Science Foundation (NSF) and the Japan Society for the Promotion of Science (JSPS)	2 months (set dates)	www.nsf.gov
JASSO International Student Scholarship for Short-term Study in Japan	Independent Administrative Institution Japan Student Support Services (JASSO)	3 - 12 months (flexible dates)	www.jasso.go.jp/scholarship/short_term_e.html
Fulbright Graduate Research Fellow	Institute of International Education (IIE), U.S. Department of State	12 - 18 months (flexible dates)	www.iie.org/fulbright
Monbukagakusho Scholarships	Japan Ministry of Education, Culture, Sports, Science and Technology (MEXT)	1-5 yrs (semi-flexible dates in accordance w/academic year)	www.studyjapan.go.jp/en/toj/toj0302e.html

reared fish were moved into 4 x 4 m acclimation cages at the release site for one week. Within 1-2 days, hatchery fish directly from tanks were released with conditioned fish from the acclimation cages. Researcher-initiated beam trawling (net size = width 2 m x height 20 cm x 8 mm² mesh; < 5 m depth) was conducted 1-2 times per week for the next month. Longer-term recaptures were supplied by fishermen (set [fyke] net, shrimp trawl and recreational fishermen [Fig.1]). All ethanol-preserved

recaptures were examined for markings, measured for growth, and subjected to dietary analyses (discussed elsewhere). Recapture rates, locations, and movements were documented.

Marking

Hatchery-reared Japanese flounder exhibit a high incidence (> 95%) of black, spotty malpigmentation on their blind (abocular) side (Tominaga and Watanabe, 1998), which acts as a natural marker of a

放流したヒラメを見つけて下さい

- ・12cmのヒラメ稚魚を甲ヶ崎へ放流しました。
- ・放流したヒラメは、囲い網の中で放流する環境に慣れさせた囲い網群と、飼育していた水槽でそのまま育成した水槽育成群の2群です。
- ・2群ともそれぞれ異なる焼き印標識をしています。



放流した場所もここです

囲い網群(背側焼き印 3点): 8,200 尾

水槽育成群(腹側焼き印 3点): 4,500 尾

・このような焼き印が付いたヒラメを見つけた方は下記までご連絡下さい

小浜観望漁業センター 0770-52-2660 (担当 藤本)

Fig. 1. Poster distributed to recreational fishermen in eastern Obama Bay. Note the series of marks denoting conditioned (dorsal) and non-conditioned (ventral).

stocked fish. Condition status was determined using two methods: (1) a series of burn marks or brands inflicted on each fish's blind side (Fig. 1), and (2) by soaking fish en masse in an alizarin complexone

(ALC) dye bath during rearing. Two ALC ring marks on the otolith (i.e., via two immersions) denoted a conditioned fish, whereas one marked a non-conditioned fish.

Study Sites

Wakasa Bay is located in central Honshū along the north coasts of Fukui and Kyoto prefectures facing the Sea of Japan (Fig. 2). We focused releases in the western portion of Wakasa Bay. In 2008 and 2009, approximately 40,000 and 80,000 10-cm juveniles were released in Takahama Bay (35°N29'38", 135°E32'44"). In 2010, approximately 13,000 12-cm juveniles were released into the eastern portion of Obama Bay (35°N31'59", 135°E45'17"). Releases in Takahama Bay and Obama Bay occurred along the sandy coastline in waters 1-2 m deep. Both bays deepen into muddy-bottomed centers towards the mouth; Obama Bay depths increase to over 20 m providing appropriate habitat for a shrimp-trawl fishery.

Results

Fish Locations and Movement

In 2008, there were no researcher-initiated recapture attempts via beam trawl; recaptures were collected solely via local fishing (set-net) efforts and market landing data from the Takahama Bay fishermen's co-op. Nine set-nets were distributed coastally around Takahama Bay (Fig. 3a). Conditioned fish were released on July 3 and non-conditioned fish were released the following morning. The first recaptures (one conditioned and one non-conditioned fish) occurred four days after release. It took five

days for the first recaptured (conditioned) fish to move 4.5 km to the opposite (western) side of the bay. Within the first month of release, conditioned fish slightly led the advancement (by 1-2 days) towards the mouth of the bay. However, the mouth of Takahama Bay is wide, and we had no way to monitor the vast expanse of deeper waters in the middle of the bay. Our last confirmed fish location was of a no-conditioned fish at the mouth of the bay on October 13th.

In 2009, researcher-initiated beam trawling was conducted via local fishermen boat charter starting the day after all fish were released. Conditioned fish were released on June 29 and non-conditioned fish were released the following morning. The first recaptures (eight conditioned and 17 non-conditioned) occurred during initial beam trawl sampling on July 1. That year, both conditioned and non-conditioned fish appeared to disperse equally (Fig. 3b). After one week, both conditioned and non-conditioned fish were detected 3.5 km across the bay. Fish captured near the release site, which were mostly no-conditioned fish, showed very little in their stomachs even up to one month after release. Our last confirmed location was of a no-conditioned fish in the lower bay on November 12th.

In 2010, the release site was moved east to Obama Bay (Fig. 3c), closer to the hatchery. Unlike Takahama Bay, Obama Bay has a very tapered mouth, the deeper waters of which are utilized

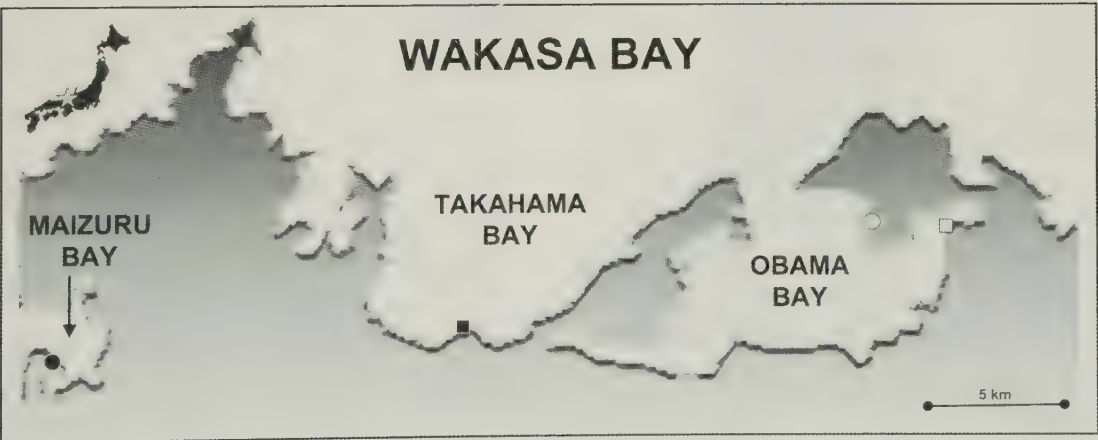


Fig. 2. Study sites in Wakasa Bay, central Honshū Japan. Black square marks the release site in Takahama Bay (2008, 2009) and white square marks the release site in Obama Bay (2010). Black circle denotes Kyoto University's Maizuru Fisheries Research Station, and white circle denotes the National Center for Stock Enhancement, Obama Station.

by a substantial shrimp trawl fishery. Out of a fleet of 10 trawlers, one was hired to collect and preserve all Japanese flounder bycatch until the end of the season in November. In addition, since there are no set-net fishermen in Obama Bay, five stationary fish traps were set up near the release site. Researcher-initiated beam trawling began three days after release. All fish were released on July 6, and the first recapture (one conditioned fish) was collected the morning after release in the fish trap nearest to the release site. Two weeks after release, the first conditioned fish began appearing in shrimp trawls approximately 5.5 km away. Again, fish captured near the release site were mostly non-feeding, non-conditioned individuals. The number of recaptured conditioned fish (41) quickly surpassed the number of non-conditioned fish (13) captured via shrimp trawler. Our last confirmed location was of a conditioned fish caught by shrimp trawler on November 20th.

Recapture rates

If we examine the overall total recapture rate for all three years combined (including researcher-initiated beam trawling; Table 2), recapture rates for conditioned fish were slightly less than those for non-conditioned fish (0.0025 as compared with 0.0026). However, since the goal of Japan's flounder stocking effort is to overcome recruitment limitations by augmenting natural juvenile supply, and thus optimizing fishing harvest (Yamashita and Aritaki, 2010), total fishermen recapture rates provide a much more practical assessment of conditioning success. Focusing on fishermen's recapture efforts and market landing data, acclimation cage-conditioned fish were recaptured more than non-conditioned fish over all three years combined (recapture rate = 0.0020 conditioned, 0.0017 non-conditioned). In Takahama Bay in 2008, conditioned fish (0.0021) were recaptured more than non-conditioned fish (0.0018), but that trend was reversed in 2009 (0.0013 conditioned, 0.0015 non-conditioned). In 2010, recapture rates of conditioned fish almost doubled that of non-conditioned fish (0.0056 and 0.0031 for conditioned and non-conditioned). Market landing data will continue to be collected.

DISCUSSION

Conditioned fish exhibited higher overall performance than non-conditioned fish in terms of movement and fishermen's recapture rates. Sparrevohn and Støttrup (2007) investigated the effects of transferring turbot (*Psetta maxima*), to enclosures at the release site six days prior to release and found that conditioning had a positive effect on flatfish survival: mortality of cage conditioned fish was half that of non-conditioned fish. Kellison *et al.* (2003) found that hatchery-reared fish released in cages showed no difference in habitat-specific growth rates compared with wild fish. Released hatchery-reared flatfish have been shown to have lower residence time than their wild counterparts (Kellison *et al.*, 2003), but site fidelity can be increased by transferring fish to *in-situ* acclimation cages before release (Fairchild *et al.*, 2008b). Conditioned fish in our study moved more actively toward the mouths of the bays than non-conditioned fish, but this may be a result of (1) high coastal water temperatures (up to 30°C) in the shallows of the release area during the summer release time causing the fish to move toward cooler, deeper water, and (2) potentially higher concentrations of prey in the deeper waters of the bay, e.g. shrimp, which form the basis of the trawling industry in the middle of Obama Bay.

Given the empty stomachs of many of the fish recaptured near the release site, our work indicates that slow boat speed (< 1 knot) beam trawling efforts may target weak, non-feeding, unmoving fish. Non-conditioned fish, mostly non-feeding individuals, were caught more often by beam trawl than conditioned fish each year when researcher initiated recapture efforts were conducted. Similarly, Sparrevohn and Støttrup (2007) found that the catchability of non-conditioned turbot caught by beam trawl was 10% higher than that of cage conditioned fish. Efforts and money for recapture may be better spent with more local fishermen involvement, especially considering shrimp trawlers tow faster (approximately 3.5 knots), use a larger net (width 6 m x height 4 m x length 16 m, 18 mm² cod-end mesh), and are capable of surveying deeper (> 20 m) waters.

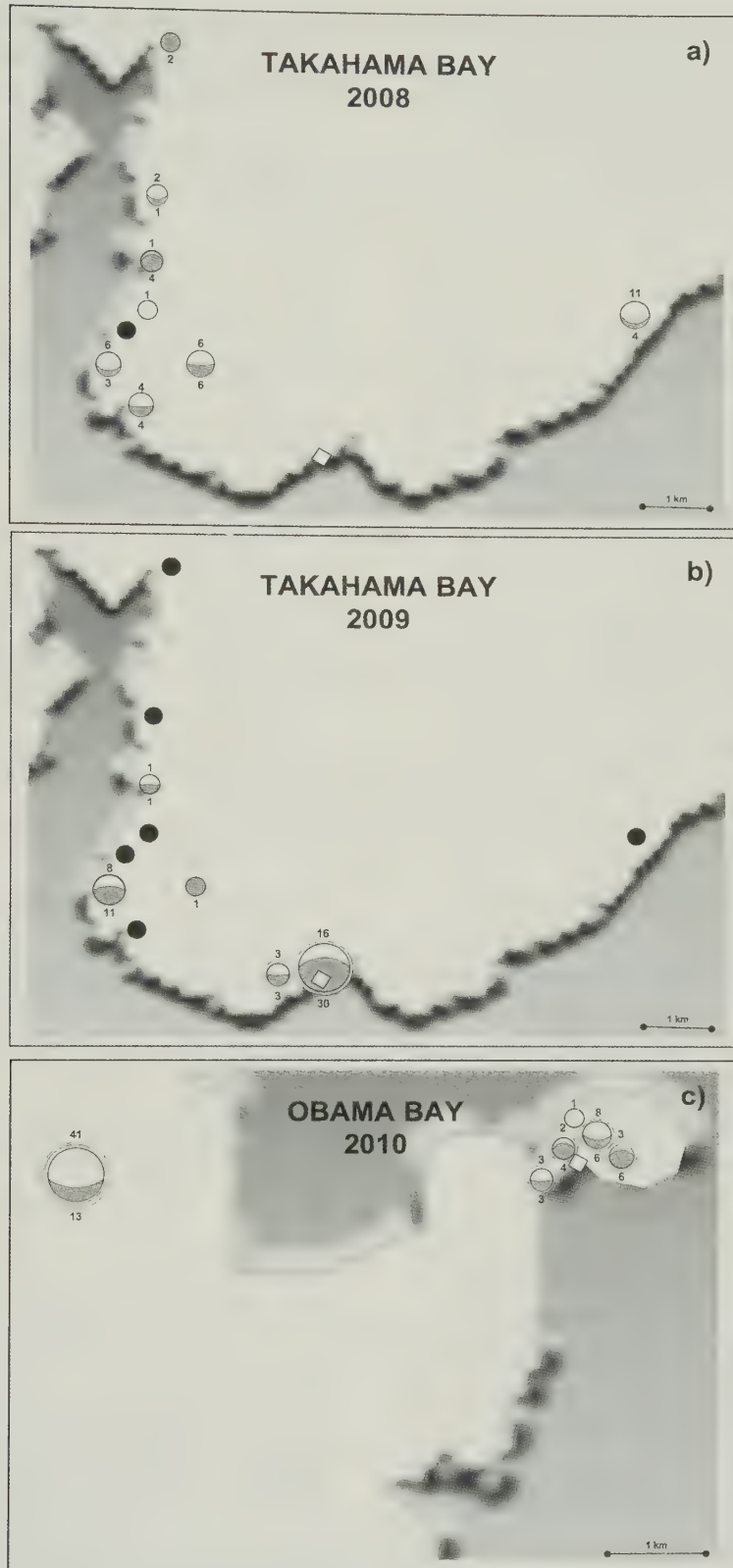


Fig. 3. Recapture locations of hatchery released Japanese flounder in (a) Takahama Bay in 2008 and (b) 2009 and (c) Obama Bay in 2010. Single-ringed circles denote fish recaptured by set-nets or traps, double-ringed circles by beam trawl, and triple-ring circles by shrimp trawler (with the exception of one fish caught in the trap nearest to the release area in 2010, which was included in the beam trawl tally nearest to the release site). Size of the circle reflects number of fish recaptured at each location. Degree of shading of the circle (white = conditioned; gray = non-conditioned) reflects the ratio of conditioned to non-conditioned fish recaptured at each location with numbers on the tops and bottoms of circles indicating actual numbers of conditioned and non-conditioned fish recaptured, respectively.

Table. 2. Release/recapture data for acclimation cage Japanese flounder stocking efforts 2008-2010 (last updated December 31, 2010). Total recapture includes researcher-initiated beam trawling. Total fishermen recapture includes all fishing and passive trap recaptures (includes market landing data), but does not include researcher initiated beam trawling

YEAR	CONDITION STATUS	RELEASE		TOTAL RECAPTURE			FISHERMEN RECAPTURE			LAST RECAP DATE
		# release	% release	TOT # recap (includ. research beam trawls)	TOT # recap %	TOT recap rate	TOT # recap fishermen (setnet, trap, shrimp trawl, rod&reel, market)	TOT # recap fshmn %	TOT recap fshmn rate	
2008	conditioned	23,400	52.35	49	56.32	0.0021	49	56.32	0.0021	08/04/10
	Non-conditioned	21,300	47.65	38	43.68	0.0018	38	43.68	0.0018	10/14/09
	total	44,700		87		0.0019	87		0.0019	
2009	conditioned	40,500	52.39	71	44.38	0.0018	52	48.15	0.0013	12/26/10
	Non-conditioned	36,800	47.61	89	55.63	0.0024	56	51.85	0.0015	12/26/10
	total	77,300		160		0.0021	108		0.0014	
2010	conditioned	8,200	64.57	59	64.13	0.0072	46	76.67	0.0056	11/20/10
	Non-conditioned	4,500	35.43	33	35.87	0.0073	14	23.33	0.0031	10/19/10
	total	12,700		92		0.0072	60		0.0047	
OVERALL	conditioned	72,100	53.53	179	52.80	0.0025	147	57.65	0.0020	12/26/10
	Non-conditioned	62,600	46.47	160	47.20	0.0026	108	42.35	0.0017	12/26/10
	total	134,700		339		0.0025	255		0.0019	

In 2009, 80,000 fish were released, the highest number of all three years, yet that year was the worst with respect to fishermen recapture rate. Successful stocking endeavors require post-release densities below the carrying capacity of the release environment (Munro and Bell, 1997). The large number of fish released into Takahama Bay at one location may have strained the immediate carrying capacity of the environment, resulting in low overall recapture. Indeed, both Tanaka *et al.* (2005) and Sparrevohn and Støttrup (2007) found that during years of higher release numbers, the fraction of recaptured flatfish with food items in their stomachs

was lower than in years when fewer numbers were released. The sudden increase in the number of flatfish predators at the release site may cause short-term, density-dependent ecological changes in the dynamics of the prey species, e.g., prey numbers as well as the behavior of prey (Sparrevohn and Støttrup, 2007), and those changes may affect the overall success of the stocking effort.

Acclimation conditioning cages may provide flatfish released for stock enhancement with time for wild behavioral adjustment, which may increase burying and feeding ability and decrease predator mortality (Sparrevohn and Støttrup, 2007).

Nevertheless, conditioning cages in themselves may attract predators simply by providing structure to a barren bottom (Fairchild *et al.*, 2008a). Swimming crabs (*Portunus gladiator*), confirmed predators of juvenile Japanese flounder (Saitoh *et al.*, 2003), were observed crawling on the outside of conditioning cages in Obama Bay and around the release site in Takahama Bay; however, the number of crabs observed was much fewer than that of the green crabs (*Carcinus maenas*), observed in Fairchild's study (pers. obs).

Japan affords invaluable opportunities for flatfish stock enhancement research that at this time is not yet feasible in the U.S. The size and scope of Japanese releases far surpasses U.S. experimental releases, which do not provide a large enough release or recapture sample for full detailed analyses of the success of an *in-situ* stocking or conditioning effort. U.S. fisheries managers and scientists, therefore, can regard Japanese flatfish stocking efforts as case studies from which to model and base their own developing flatfish stocking programs.

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[from original abstract submission]

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Acoustic Conditioning and Ranching of Black Sea Bass *Centropristis striata* in Massachusetts USA

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Abstract : Acoustic ranching consists of training fish to school to an area via a sound stimulus that is coupled with a food reward (classical Pavlovian conditioning). It may present an opportunity to raise fish with less environmental impact and at less expense than typical open ocean fish farms. Some advantages include (i) low capital and operating costs to construct, install, and maintain a feeding and recapture station, (ii) low feed costs because fish have opportunities to forage on wild food as well as formulated diets, (iii) low impact on the environment due to natural dispersion of fish and their wastes, and (iv) the technology could aid stock replenishment efforts by weaning hatchery-raised fish from pelleted diets to feeding for themselves in the wild.

This project represents the first attempt to farm marine fish with acoustic ranching in North America. In June 2008 we erected and installed an AquaDomeTM, a 10 m wide by 5 m high geodesic dome in Buzzards Bay, Massachusetts USA. The AquaDomeTM was fitted with a feeding tube, an underwater speaker, and underwater cameras to monitor and record fish behavior. Approximately 5,000 tagged black sea bass (50 to 80 g) were stocked into the AquaDomeTM. The fish were trained in the cage by feeding them twice a day in tandem with a sound cue. Once the training was completed, 2.54 cm mesh on the AquaDomeTM was replaced with 10.16 cm mesh so the fish could swim out and back in when cued to sound. Many set up residency on the nearby rocks. We conclude that fish have longer memories than previously thought and are readily adaptable to acoustic training. However, the application of this technology in the field is fraught with risks, especially when there is the threat of predators.

Key words : black sea bass, acoustic conditioning, aquaculture, sea ranching, *Centropristis striata*

Introduction

Open Ocean Farming Overview: Despite a decade of research and development and technological advancements, open ocean fish farming has not yet demonstrated a profitable future in the continental US. (Kite-Powell *et al.*, 2003; Posadas and Bridger, 2004). There are some bright spots in our tropical

territories (e.g., Puerto Rico and Hawaii) with uniquely appropriate species such as cobia (fast growth) and pacific threadfin (high local demand and price). But experience and economic models have shown that the profitability of open ocean aquaculture in the Gulf of Mexico may only be viable with an exceptional species such as cobia (Posadas and Bridger, 2004). For open ocean

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fish farming to become economically and widely successful, the high expense of farming on the high seas needs to be significantly reduced. About 10% of the annual expenses associated with open ocean fish farming relate to the capital cost of submersible cages and moorings, and about 8% are for operating expenses, including insurance and maintenance according to a variation on a cage-based economic model (Kite-Powell *et al.*, 2003). Another 50 to 60% is associated with feed and supplying feed. If these expenses can be reduced, a variety of fish species in coastal environments could be ranched economically. Our economic modeling suggests that recapture rates of 55 to 70% of ranched fish could compete with open ocean cage culture operations depending on the amount of supplemental natural forage consumed by ranched fish. Our project tested methodologies that could significantly reduce these expenses by eliminating the capital and maintenance cost of the cage and potentially stressful confinement of the fish, and by allowing the fish to supplement their diet with natural forage.

The commercial future of open ocean finfish ranching as proposed here will depend on its economic viability. Prior work on the economics of ocean ranching has focused primarily on stock enhancement perspectives, particularly for salmon (Stokes, 1982; Whitmarsh, 2001). Other studies describe ocean ranching of cod in Norway (Midling *et al.*, 1993) and sea bream in Japan (Okamoto, 1982). In addition, there is some recent (in some cases unpublished) work on the economics of ocean ranching efforts in Japan (Nakahara R., personal communication; Nakahara, 1992). In this project, we examined the economics of open ocean finfish ranching of black sea bass in New England from both the public stock enhancement and the private commercial production perspectives.

Acoustic Conditioning of Farmed Fish: Operant conditioning is a widely understood phenomenon made famous by Ivan Pavlov and his experiments with dogs that salivated at the sound of a bell in anticipation of food. It requires providing a reward (e.g. food) for a desired response to stimulus. Training fish to school to an area known to be associated with feeding via a sound stimulus that

is followed shortly by feeding (hereafter referred to as acoustic conditioning) is a well established protocol and was first documented 40 years ago with trout (Abbot, 1972). Sound has broad efficiency in attracting fish that have been appropriately trained with operant conditioning procedures (Yan and Popper, 1991). It has been used as a means to enhance feeding response in larval and juvenile Atlantic cod (Oiestad *et al.*, 1987), for cageless ranching of cod in Norwegian fjords (Midling *et al.*, 1993), and as a means to feed wild cod populations in Iceland (Bjornsson, 1999; 2002). In all cases, cod of various ages were successfully conditioned to feed in association with an auditory stimulus. In Japan, acoustic conditioning and ranching have been used by several prefectural fisheries to help acclimate, reduce stress and keep newly released sea bream (*Pagrus major*) and striped jack (*Pseudocaranx dentex*) in specific habitats that are being restored (Okamoto, 1982; Kuwada *et al.*, 2000). In three experiments on acoustically conditioned and released juveniles around a net cage/feeding station, 85 to 95% of the fish were recaptured (Masuda and Tsukamoto, 1998). Willis *et al.* (2002) used acoustic conditioning with grass carp in a laboratory setting to test the utility of sound to attract fish for recapture. They predicted that 94% of the fish could be recaptured in lake management settings. Acoustic conditioning for fish ranching has not been attempted with marine fish in North America.

Background and Rationale for Black Sea Bass

Aquaculture: Black sea bass (*Centropristis striata*; hereafter referred to as BSB) have been the focus of research and development over the last decade in the Northeastern USA (University of New Hampshire; University of Rhode Island; Massachusetts Institute of Technology; National Marine Fisheries Service Milford Laboratory; Great Bay Aquaculture, New Hampshire) and the Southeastern USA (University of North Carolina-Wilmington; Georgia Sea Grant; Harbor Branch Oceanographic Institute, Florida; and Southland Fisheries, South Carolina). We tested the ranching concept with BSB since this species is a good candidate for a number of reasons:

1. Fingerlings are available from commercial hatcheries.

2. Techniques for breeding and larviculture of the species have been researched and well described.
3. BSB grow relatively fast and under optimal culture conditions; juveniles suitable for stocking for open ocean ranching can be reared to market size in six months (May-October; Copeland *et al.*, 2002).
4. BSB are important recreationally and commercially with some markets having a very high return (e.g., fresh fish market price: US\$11.00/kg, live fish price US\$18.70/kg). The 2009 dockside price for BSB was US\$5.50/kg (<http://www.st.nmfs.gov/st1/commercial/index.html>).
5. BSB are native to New England and tend to be seasonally residential around bottom structures like reefs and outcroppings; such locations would provide shelter for ocean-ranched fish and could also provide habitats for fisheries enhancement.

Objectives

Our goals for the two-year study were:

1. Develop acoustic conditioning methods for black sea bass so they will respond to feeding on prepared diets in a predetermined space for possible recapture, even when given opportunities to feed on natural forage, and test their memory to respond.
2. Complete field tests to grow acoustically conditioned BSB released in open waters without confinement and document the growth and recapture rates of BSB.
3. Develop an understanding of the economics associated with this project and these methods as models of open ocean finfish ranching and stock enhancement.

Materials and Methods

Determining an Effective Acoustic Conditioning Protocol for BSB and Limits of Memory: Twenty-five BSB (20 g to 25 g each) or 30 BSB (15 g to 20 g each) were placed into replicate round 250 L tanks, 1.25 m in diameter (six tanks total). The tanks were cordoned off using black plastic sheeting to isolate the BSB from any visual stimuli outside the tank. A semicircular barrier with a 10 x 25 cm opening (entrance) was placed into the tank (Figure 1). The area within the barrier was where all feeding occurred during conditioning and was referred to as the feeding zone. A 20 watt 4 ohm underwater speaker was suspended within the feeding zone and a PVC pipe was positioned above it through which feed pellets could be delivered into the feeding zone from behind the black plastic sheeting out of view of the fish. The speakers were connected to a Radio Shack MPA 20 watt amplifier and a Tenma 72-505 Audio Generator was used to produce a pure 280 Hz

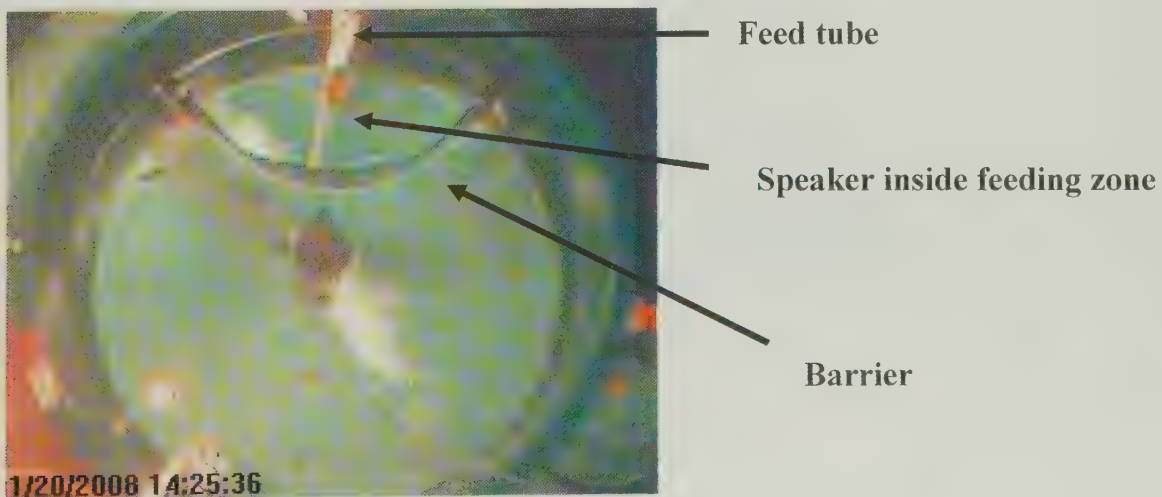


Fig. 1. Typical experimental tank set up (birds-eye view).

tone in the feeding zone. Trials were observed and recorded using 6 CCD video cameras (1 over each tank) and a computer equipped with a 120 fps (20 fps per camera) 16 channel GeoVision GV-800 DVR card with GV-800 surveillance software.

For our standard conditioning protocol, a 280 Hz tone was generated underwater for 20 seconds and then the tone was stopped for 10 seconds before feed pellets were delivered into the feeding zone. This procedure was continued three times a day with feed delivered at a rate of 1.5% body weight per day. When at least 80% of the BSB responded to the tone alone (before feeding) and moved into the feeding zone in at least one of the three daily feedings for 10 consecutive days, we considered that tank population conditioned for our study purposes.

Once the initial conditioning was completed, fish in three replicate tanks were subjected to alternating days of feeding inside (with acoustic stimulus) and outside (without acoustic stimulus) the feeding zone. This was increased to one day with acoustic conditioning and two days without acoustic stimulus for up to 15 days. The number of intervening days without acoustic stimulus increased as long as >80% of the fish responded on those alternate days with acoustic conditioning. When the number of fish responding fell below 80%, reconditioning was attempted for two more consecutive days. If response remained below 80% for all three daily trials at the end of three consecutive days, the trial was stopped, but if the response exceeded 80% then the tank was subjected to the next incremental number of days between reconditioning days.

Methods for the Field Trial:

We purchased 5,000 two to three gram fingerlings from Great Bay Aquaculture in August, 2007 and grew them to 75 gram stocking size by July, 2008. Depending on the speed with which we needed to grow them to meet that target, we selected a culture temperature between 15 and 25°C (Cotton *et al.* 2003). Fingerlings were stocked in several 3.05 and 3.66 m diameter round tanks as they grew. The final density was <10g/L. Fish were fed a standard commercial marine fish feed to apparent satiation twice a day. Water quality was monitored at least weekly to maintain dissolved oxygen at >8 ppm and

acceptable ammonia and nitrite levels. Growth rates were tracked with monthly samples of weight and length. Once the fish reached 50 g on average, we tagged them with floy tags marked with a phone number and the word reward.

We selected a site near the northern most Weepecket Island in Buzzards Bay (N41°31' 33", W 70°43' 85"; Figure 2). The site is a flat seabed about nine meters deep (mean low tide) and about 10 meters from the margin of the rocky outcropping that composes the island. The substrate at the site is bare sand with some fine silt on top. In June, 2008 we installed an AquaDome™ structure for facilitating the acoustic conditioning, controlled fish release, occasional feeding, and potential recapture for census and survival estimates. The AquaDome™ is an aquaculture containment structure based on the commercial Aquapod™ fish cage manufactured by Ocean Farm Technologies LLC of Searsmont, Maine. The structure consisted of a hemispheric portion of a geodesic sphere (10 m in diameter and 5 m high) with a replaceable mesh cover. It was secured to the sea floor bottom by five 544 kg deadweight anchors and line (Figure 3). Additional modifications included a lighted marker buoy connected to a 7.6 cm feed hose that penetrated the AquaDome™ and extended 1.83 m from the bottom.

Once the fish were stocked in the AquaDome™, we fed them via acoustic conditioning daily. Underwater sound was generated by a commercial grade Lubell transducer and amplifier. Three different types of underwater video cameras were fitted with connectors (suspended on the surface buoy) so we could monitor the inside the AquaDome™. The cameras were linked to a four channel DVR with a flash memory card for storing footage as well as a small video screen for field monitoring. Footage was downloaded to a computer for quantifying fish behavior. Feed quantity was based on video observations of feeding response (and compared to expected consumption based on percent body weight per day feeding rates (Copeland *et al.* 2002). The effectiveness of video monitoring was confirmed by periodic observations by SCUBA divers.

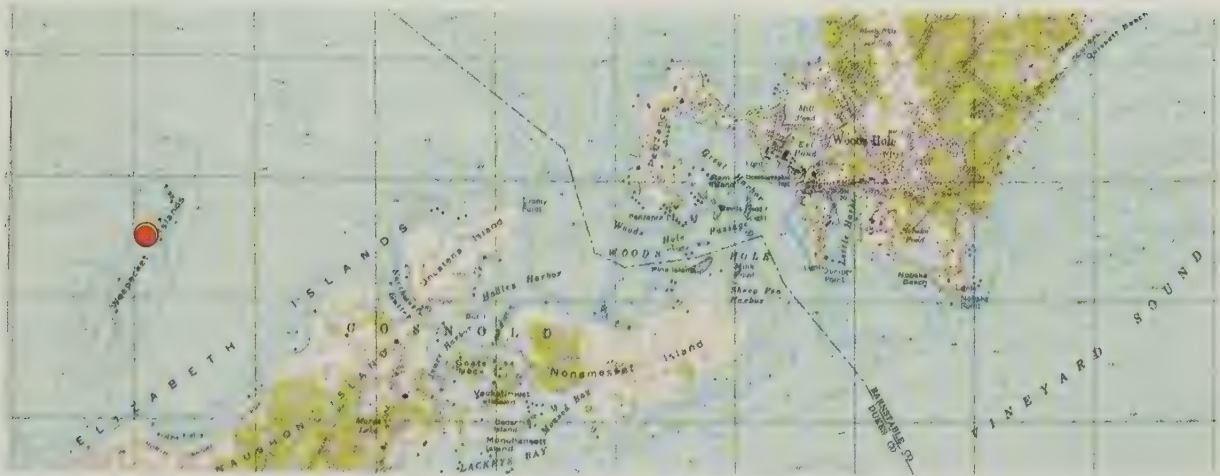


Fig. 2. Field site near Weepeket Islands (left) 3 nautical miles from Woods Hole.

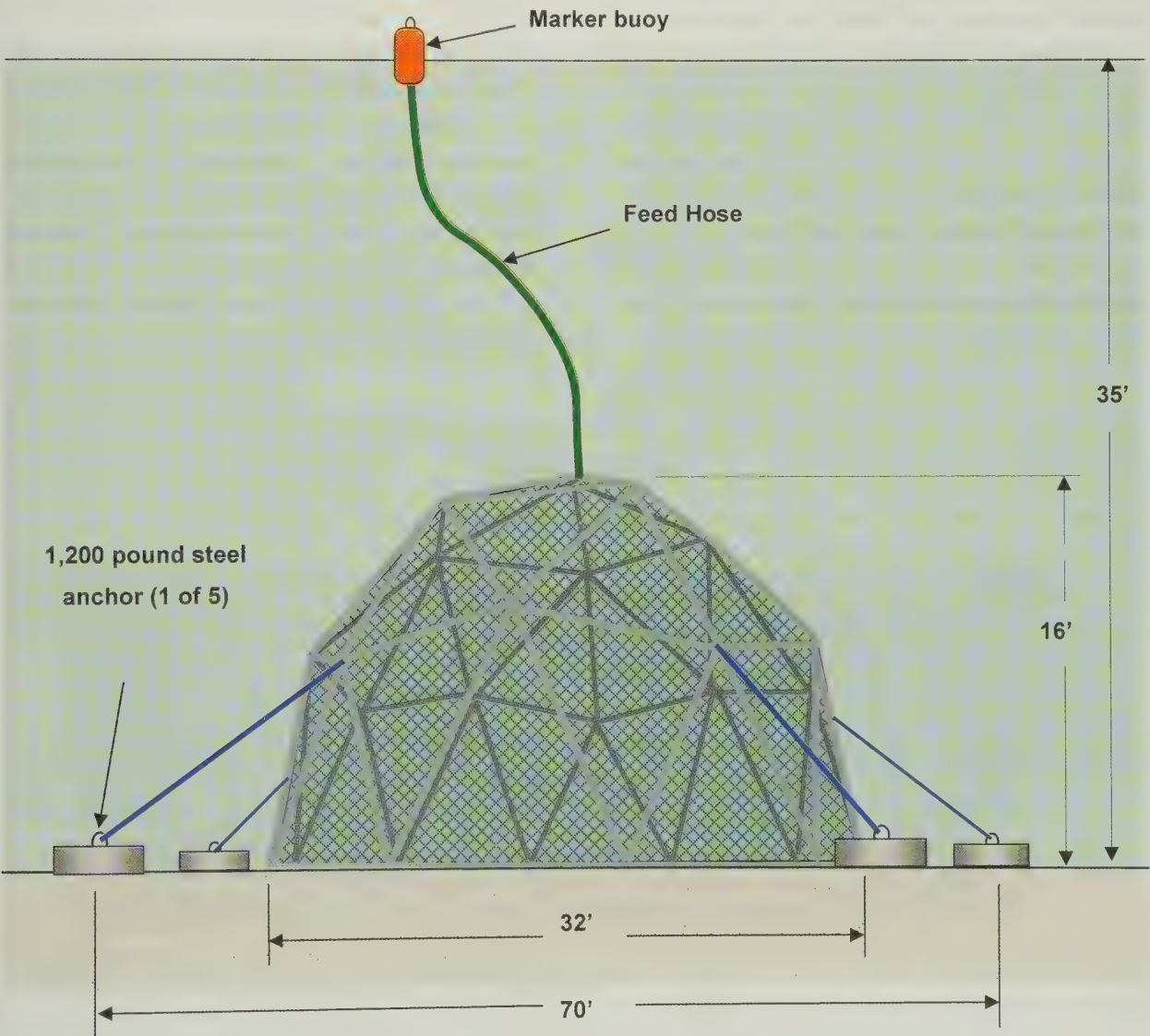


Fig. 3. Schematic of AquaDome™ and mooring setup in Buzzards Bay, MA.

Results and Discussion

Effectiveness of Acoustic Conditioning and Memory Response: Training trials typically followed the example below with slow response during the first week, improving response in the second week and steady response after two weeks (Figure 4). In our memory trials, two replicates performed almost identically with responses well over 90% after two and three days of no conditioning. However, after five days of no acoustic stimuli and conditioning, the response of both replicates dropped well below 90% (between 17% and 48%), and after three consecutive days of reconditioning their responses remained below the 80% required to graduate to a longer period of no conditioning. On the other hand, a third replicate responded well after a five-day absence of conditioning with daily averages well over 80% (Figure 5). They were then subjected to a seven-day absence of conditioning after which they attained a single trial response of 92% (66% average for that day). They were then subjected to 10 days with no conditioning and an additional seven-day no conditioning period. After the 10-day and seven-day conditioning lapse they required three days of conditioning to successfully attain the >80%

response required.

The differences between the performance of the first two replicates and the last may be attributed to the amount of time on the conditioning regimen leading up to the trials. Conditioning of the first two replicates was started at the same time, and the last replicate had a full week longer conditioning period. The fact that the last replicate could withstand longer lapses in acoustic conditioning suggests that perhaps the length of time that BSB have been conditioned is correlated to the length of time they will remember. We can conclude that populations of fish retain good memory of the conditioned response for at least three days. Longer conditioning periods prior to memory testing may lengthen memory retention up to 7-10 days.

Field Trial and Assessing the Results from July 2008 to December 2008: From when the fish were stocked in the AquaDome™ on July 17, 2008 until their release on September 3, the bass were exposed to 47 days of training to tone and feed, sometimes twice a day. On September 4 we finalized the replacement of two 2.3 m² sections of 2.54 cm mesh with 10.16 cm mesh on the AquaDome™ so the fish could swim in and out. On September 5 we conducted a

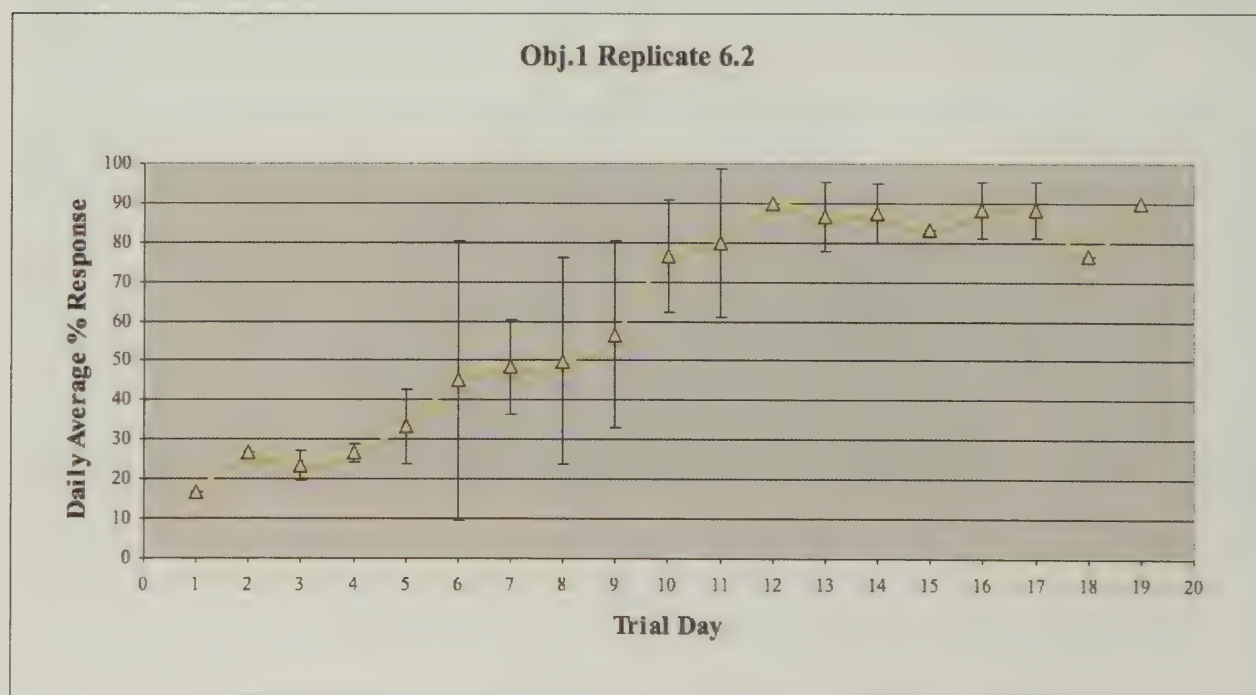


Fig. 4. Example of typical fish population response to initial conditioning and training protocol.

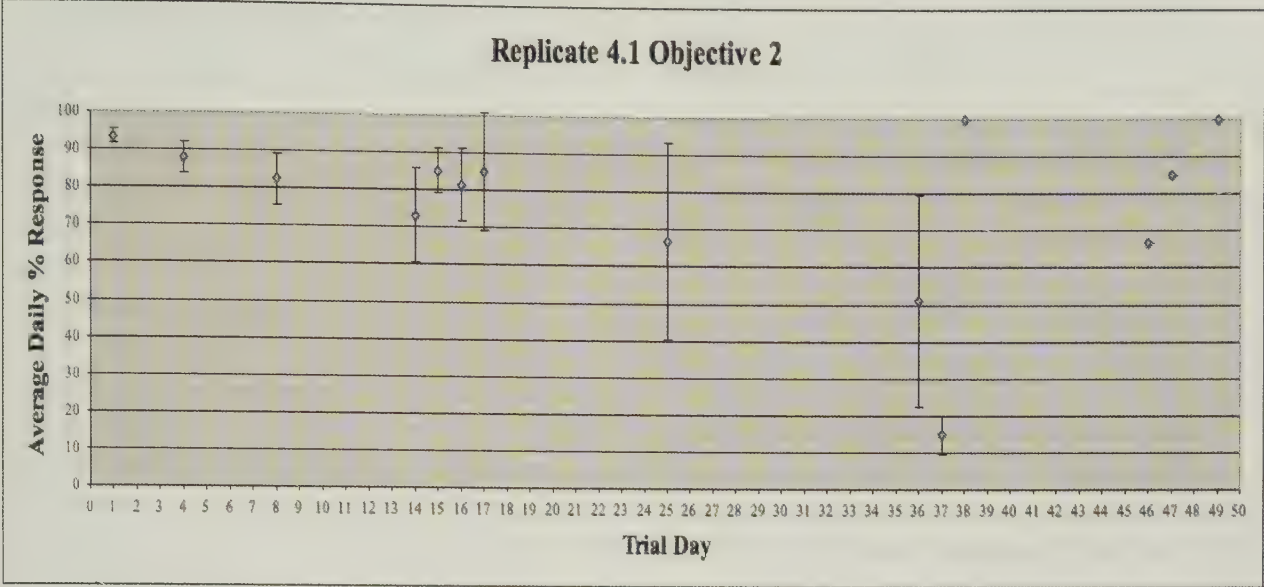


Fig. 5. Example of memory capabilities of a fish population as measured by acoustically conditioned feeding response interspersed with extended intervals of feeding without sound.

typical tone/feeding and the fish responded with typical interest and appetite. We then did not feed the fish for two days to encourage them to emigrate and begin to forage on their own. On September 12th, with observations by SCUBA divers we conducted a tone and feeding with typical response inside the AquaDome™ but few fish outside moved to it. We conducted a survey in the surrounding rocky habitat and found hundreds of BSB scattered to within 100 m of the Dome, but the majority of them remained within or immediately surrounding the Dome.

To encourage further emigration of the BSB, there was no feeding conducted for three successive days. On 16 September the response to our tone/feeding was remarkably poor. When we reviewed the video recording we noticed one of the camera views showed a predatory bluefish inside the Dome and other bluefish swimming around the Dome in a menacing fashion. Divers entered the Dome that afternoon to evict the bluefish which appeared to be too large (approximately 3 to 4 kg.) to have entered through the mesh. Examination of the Dome did not reveal other possible points of entry at that time though sand seemed to be eroding from under one side. Anchor lines had loosened and peak tides may have tipped the Dome to create a gap large enough

for the bluefish to enter.

On subsequent feeding days and diver observations we did not see the robust feeding that we had expected. Via our cameras we continued to see bluefish swimming around the AquaDome™ that presumably discouraged the BSB from responding to food. We set fish traps among the rocks and made dive observations of hundreds of BSB but failed to draw them back to the AquaDome™. We sacrificed some trapped fish and noted fresh prey in the stomach of some of them — a good sign of adaption to the wild. By mid-October water temperatures were dropping, and we found it very difficult to trap more than couple BSB a day. This was expected since it was the season for the species to migrate to warmer waters for the winter. We hope to document some successful contribution to the local fishery from our release of the fish via tag returns in the years to come when they are legal harvest size.

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Artificial Completion of the Japanese Eel, *Anguilla japonica*, Life Cycle: Challenge to Mass Production

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Abstract : Current eel culture depends entirely on glass eels captured from the wild. However, in recent decades, eel populations have declined. Thus, establishment of the technology for producing sustainable supplies of eel seeds is required. To achieve that end, selective breeding and mass production of glass eels is necessary. This year, we were successful in closing the Japanese eel life cycle. However, we have not as yet established techniques for mass production of glass eels because of various technical difficulties. In this paper, we describe the significance of closing the eel life cycle and the challenges that need to be overcome in order to develop a system of glass eel mass production.

Key words : ell culture, glass ell production, ell life cycle, *Anguilla japonica*

The mysterious life cycle of eels has attracted many researchers. For a long time, no one could find eel eggs or larvae in the habitats where the adults were found; such as rivers, ponds, coastal waters. Early in the 20th century, Schmidt (1922) conducted numerous expeditions and discovered that the spawning area for both the European eel (*Anguilla anguilla* [L.]) and American eel (*Anguilla rostrata* [LeSueur]) was located far offshore in the Sargasso Sea region of the Atlantic Ocean. In 1991, nearly 70 years after Schmidt's (1922) discovery, the Philippine Sea of the western North Pacific was determined to be the spawning area of the Japanese eel, *Anguilla japonica* Temminck & Schlegel. Thus, the life cycle of anadromous eels was gradually uncovered (Tsukamoto, 2009).

Populations of *Anguilla japonica*, *A. anguilla*, and *A. rostrata* have decreased markedly in recent decades due to overfishing, environmental destruction, or other unknown factors (Casselman, 2003; Dekker, 2003; Tatsukawa, 2003). On the other hand, establishment of Japanese eel culture has

maintained the availability of eels to consumers at reasonable prices. However, current eel culture depends entirely on glass eels captured from the wild. A decrease in the availability of glass eels and increase in demand for eels in the marketplace will inevitably lead both to increased prices and decreased natural eel stocks. The vicious spiral could be stopped through the development of techniques to mass produce glass eels through aquaculture.

History of Research for Rearing Eel Larvae and Closing of Their Life Cycle

In Japan, attempts to induce artificial maturation of the Japanese eel started in the 1960s. Yamamoto and Yamauchi (1974) were the first to successfully obtain fertilized eggs and larvae through hormone treatments, and after two-week rearing period the preleptocephalus larvae reached 7mm TL (Yamauchi *et al.*, 1976). Thereafter, other researchers succeeded in obtaining eel larvae (Satoh, 1979; Wang *et al.*, 1980), but suitable larval feeds were not identified.

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Eel larvae cannot be reared with feeds, such as rotifers, that are often used in conjunction with other finfishes. Development of a suitable diet was difficult since it began in the absence of ecological information on larval eels. Even now their food habits in nature have yet to be determined.

Tanaka *et al.* (2001) developed a slurry type diet made from shark eggs. The diet has enabled us to rear eel larvae and led to the first successful production of glass eels in captivity (Tanaka *et al.*, 2003; Kagawa *et al.*, 2005). Since then, we have made an effort to develop a captive broodstock of adult eels reared from the egg stage. That has enabled us to complete the task of closing the life cycle of the Japanese eel (Fig. 1), which was achieved in 2010. Details of the process will be described elsewhere. We have already produced more than 300 glass eels to an age of 240 days after hatching (dah) and grow them to produce the next generation. Before achieving that breakthrough, all eggs were obtained from adults either captured from natural waters or grown from captured glass eels. We determined that rearing eels from the egg in a controlled environment does not impede oogenesis in adult stage. Closure of the life cycle and selection of individuals to be reared as broodstock provide the opportunity to produce cultured eels of high quality. Selective breeding for faster growth and higher survival rates is thought to be promising at both the larval and adult stages. Of course, improvement in the taste of cultured eels will also be expected. The

next challenge will be to find a way to consistently mass produce glass eels.

Issues to Resolve for Mass Production of Glass Eel

Some technical developments have been accomplished by several researchers with respect to mass producing glass eels in captivity. Tsukamoto *et al.* (2009) discovered that cultured eel larvae have greater buoyancies than captured larvae and that their buoyancy showed ontogenetic change. Okamura *et al.* (2009a) reported that an intermediate salinity (50% of sea water salinity) can yield better growth and survival performance in the early life stages of eel larvae. Okamura *et al.* (2009b) developed a new rearing system using a planktonkriesel instead of a typical tank. Owing to these developments, survival rates of larvae have steadily improved. However, mass production of glass eels has not been realized because of the remaining technical difficulties. Statistics compiled by the Japanese government indicate that more than 21,000 tons of cultured eels are produced annually, which is equivalent to more than 10 million eels (the white paper of fisheries, Japan, 2010). Thus, there is a very long way to go before a significant contribution to the annual total can arise from cultured glass eels. The following difficulties associated with mass production must be overcome:

- 1) New feeds need to be developed,
- 2) The rearing process must be simplified,

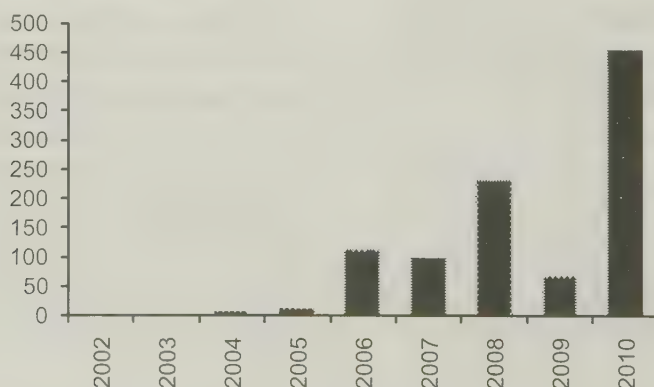


Fig. 1. Number of produced glass eels in Shibushi station. The number of metamorphosed glass eels between April to the following March of each year (to Nov. in 2010) are represented.

- 3) The rearing period must be shortened,
- 4) Diseases must be prevented, and
- 5) Morphological abnormalities must be prevented.

Today, eel larvae rearing depends entirely on a diet made from shark eggs, but natural shark populations cannot support mass production of glass eel quantitatively. In fact, spiny dogfish, *Squalus acanthias*, from which eel larval diets are formulated, was proposed for inclusion in appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; Cop15Prop18, proposed by Palau and Sweden). Therefore, we must find effective new dietary formulations. Generally, plant materials are inexpensive and easily obtainable in quantity. But, there are few examples of successful use of

plant-based feeds for rearing marine fishes. On the other hand, chicken eggs, as well as eggs from fish and other animals may be acceptable materials because they resemble shark egg in their composition. Fishmeal, which is used in many fish feeds, including those of adult eels, may have potential. Fishmeal is also available in quantity, though the price is higher than plant protein sources, in general. We have developed an "intestinal fullness index" for one of the evaluations of diet effectiveness (Fig.2, Masuda *et al.*, 2010). Using this index, we are searching for new suitable dietary ingredients.

The current larval rearing process is too laborious. Current feeding procedure is as follows (Tanaka *et al.*, 2001): Larvae are kept in the dark except during feeding time, because they swim down and

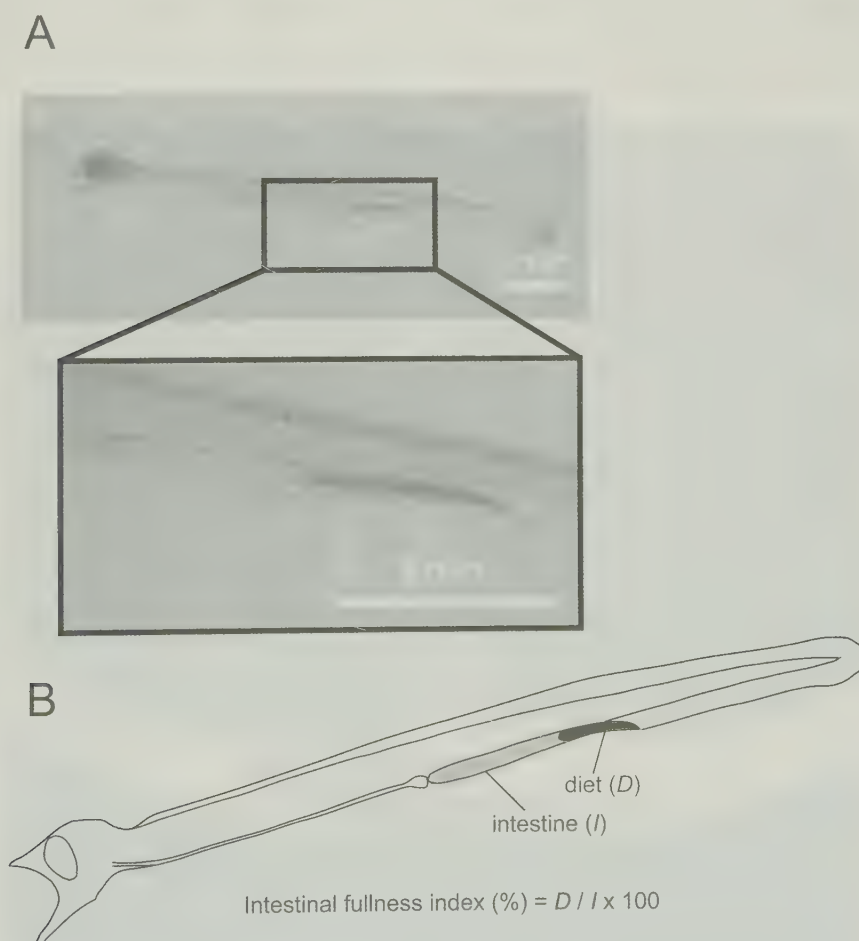


Fig. 2. A Japanese eel larva that had ingested milk (A). Intestine (gray and black) and diet (black) for calculating the intestinal fullness index(B). This figure is modified from Masuda *et al.* 2010.

push persistently on the bottom of tank in the light resulting in jaw breakage. At feeding time, we turn on a light and put the slurry diet on the bottom of tanks (Fig. 3). The larvae swim to the bottom to feed. Before putting in the feed, we must stop the water flow because they are not good swimmers. After allowing the larvae sufficient time to feed, a hose is used to flush any remaining feed from the tank, since normal water flow is not sufficient to do the job. Finally, we turn off the light. Using the described procedure, it is unavoidable for larvae to contact with walls of the tank. The inevitable mortalities can accumulate on the tank bottom, leading to bacterial growth that may induce infectious disease. Thus, we must remove dead larvae by hand. In addition, we must prepare clean tanks and transfer the larvae to them each day.

Developing a feed that is suspended in the water column may be a solution to the problems associated with feeding on the tank bottoms. A colloid-type diet

might be suitable (Masuda *et al.*, 2010). When fed milk, a typical colloid-type diet, larvae survived until 26 dah.

The period of time required for rearing eel larvae from hatch to metamorphosis is too long. Until 2009, the rearing time from hatch to completion of metamorphosis had been from 153-754 dah in Shibushi station (Fig. 4). In nature, on the other hand, larvae are thought to metamorphose into glass eels from 100-160 dah (Arai *et al.*, 1997). Thus, the larval period in captivity is not only too long, but also various much more widely than is the case for wild larvae.

The long rearing period complicates the research because of the time involved to complete each experiment. Furthermore, keeping various larval stages at the same time is apt to make the rearing environment unsuitable. In 2010, our new rearing system produced accelerated larval growth, and glass eels were obtained beginning 131 dah (Masuda

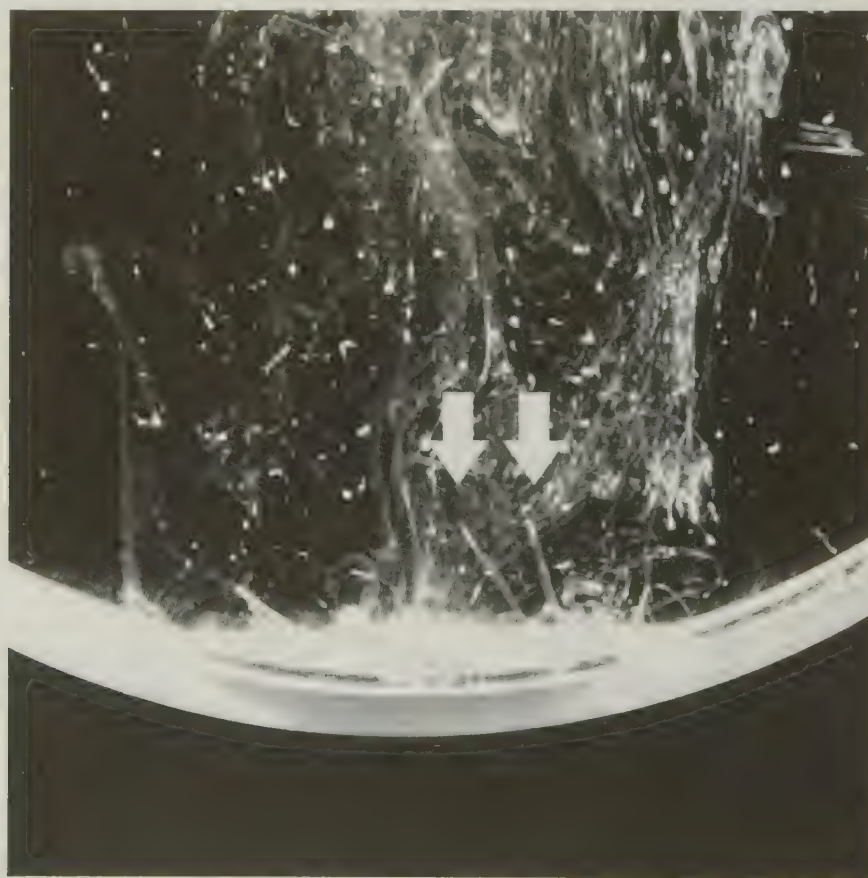


Fig. 3. Eel larvae (6 dah) eating the slurry-type diet. Larvae are pointed out by arrows.

et al., 2011). Details of the experiment will be published in near future. We hope that the results obtain in 2010 will contribute to reducing the costs of labor and energy associated with glass eel production. Moreover, shortening of rearing period can contribute to reducing the risk of diseases, morphological abnormalities, and other deleterious factors.

The danger of diseases associated with larval rearing has recently emerged as an issue. As improvements in rearing technology lead to increases larval survival rates, the relative impact of diseases increases (Fig. 5). From our observations, it seems that a disease kills larvae in a short time; only several hours in some cases; and sometimes leads to mass mortality. However, specific pathogens have not been identified. We have no idea if we're dealing with a single or multiple pathogens.

Finally, morphological abnormalities are a problem, though they are not always fatal. For example, larvae with broken jaws, the most critical abnormality, can eat the slurry-type diet, grow, and metamorphose into glass eels. However, juveniles with broken jaws cannot eat any type of food and die of starvation. Curved larvae, folded glass eels, and broken necks of glass eels are often seen (Fig. 6). Generally, such larvae and juveniles are hard to feed in spite of having normal jaws. Thus, they are at serious disadvantage with respect to survival and their growth is always poor.

Conclusion

We can produce glass eels from eggs, though the cost is very high. The next steps are to solve the remaining problems hindering mass production of high quality eel larvae. Having found a way to rear eels throughout their life cycle, it might be possible to find other added value, such as "eels for enjoying their style and color." Before that, it will necessary to establish techniques for mass production of glass eels at a reasonable cost and supply them to aquaculturists for growout. Ultimately, the hope is to supply the market with high quality and eels that meet consumer expectations in terms of flavor and cost.

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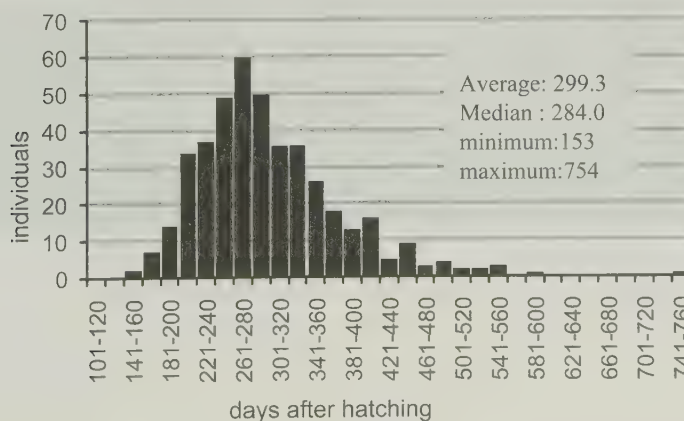


Fig. 4. Days after hatching to complete metamorphosis of eel larvae in Shibushi Laboratory. (Sum of data between April 2007 to July 2010).



Fig. 5. Larvae with pathology. Upper: Larvae (90 dah) with white head and tail. Lower: Larvae (124 dah) with broken throat.



Fig. 6. Larvae with morphological abnormalities. A: Curved larva (86 dah, left) and larvae with broken jaw (80 dah, right). B: folded glass eel (left) and glass eel with broken jaw (right).

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CONTENTS

Overview of Aquaculture Research Policy

1. Course of the Research for Sustainable Aquaculture in Japan
Fuminari Ito (National Research Institute of Aquaculture, Fisheries Research Agency) 1

Aquaculture Industry Overview and Research Planning

2. Aquaculture in the United States of America: Present Status and Future Opportunities
Paul G. Olin (UCSD, Scripps Institution of Oceanography) 7

Techniques of Aquaculture Production

3. Spawning and Larval Rearing of California Yellowtail (*Seriola lalandi*) in Southern California
Kevin Stuart (Hubbs-SeaWorld Research Institute) 15
4. Charting the Future of Aquaculture Feeds in the United States
Michael B. Rust (Resource Enhancement and Utilization Technologies Division,
Northwest Fisheries Science Center, NOAA) 23
5. Progress of DNA Marker-Assisted Breeding in Mariculture Finfish
Akiyuki Ozaki (National Research Institute of Aquaculture, FRA) 31
6. Development and Evaluation of Real-Time Loop-Mediated Isothermal Amplification
Methods for the Rapid Detection of Penaeid Viruses
Tohru Mekata (National Research Institute of Aquaculture, FRA) 39

Management, Social and Economic Issues of the Aquaculture Industry

7. The Political Economics of United States Marine Aquaculture
Gunnar Knapp (Institute of Social and Economic Research, University of Alaska
Anchorage) 51

Supporting Techniques of Aquaculture

8. Inland Marine Fish Culture in Low-Salinity Recirculating Aquaculture Systems
Marty A. Riche (U.S. Dept. of Agriculture, Agricultural Research Service) 65
9. Land-Based Poly-Eco-Aquaculture of Abalone and Seaweed in a Small Scale
Recirculating System Using a Recycled Freezer Container
Mohammad M. Rahman (Faculty of Fisheries, Kagoshima University) 77
10. Pond-to-Plate Analysis of the U.S. Farm-Raised Catfish Industry
Terrill R. Hanson (Department of Fisheries and Allied Aquacultures, Auburn University) 85

Stock Enhancement

11. Case Studies in Flatfish Stock Enhancement: A Multi-Year Collaborative Effort to
Evaluate the Impact of Acclimation Cage Conditioning for Japanese Flounder,
Paralichthys olivaceus, in Wakasa Bay, Japan
Michelle L. Walsh (University of New Hampshire) 93
12. Acoustic Conditioning and Ranching of Black Sea Bass *Centropristis striata* in Massachusetts USA
Scott Lindell (Marine Resources Center, Marine Biological Laboratory) 103

Hatchery, the Present State and Issues

13. Artificial Completion of the Japanese Eel, *Anguilla japonica*, Life Cycle:
Challenge to Mass Production
Yoshitsugu Masuda (Shibushi Laboratory, National Research Institute of
Aquaculture, FRA) 111